same, with the slope generally dipping toward the southeast. These isobases clearly depict some combination of upwarping toward the north (probably isostatic upwarping resulting from deglaciation) and crustal downwarping toward the southeast.

Our model is subject to one real constraint. The present position of the North Atlantic shoreline results from interaction of postglacial eustatic change and crustal movement. Late Wisconsin marine deposits are exposed at increasingly higher altitudes north and northeast of Boston; raised marine deposits are absent south and southeast of Boston. Ages of radiocarbon-dated samples from these Late Wisconsin marine deposits range from about 12,000 to 14,000 years (*5*, *12*). Since, according to the Redfield eustatic curve (Fig. 1), sea level was then 29 to 37 m lower than at present, the zero isobase or "hinge line" really belongs somewhere between our −20-m and −40-m isobases. Alternately, Curray's (*4*) approximate mean eustatic sea level, from compiled data for the period between 12,000 and 14,000 years ago, indicates that our −60-m isobase is the true zero isobase.

The data derived principally from Emery and Garrison's report disclose a pattern of downwarping of the edge of the continental block off the northeast coast of the United States. This downwarping probably does not represent a water-loading effect [as Bloom (*13*) suggested] because the Gulf Coast continental shelf has not, according to Emery and Garrison, undergone downwarping similar to that of the North Atlantic littoral. The scarcity of data from off the coasts of New Jersey, the Delmarva peninsula, southeastern Virginia, and North Carolina could permit an alternate construction of isobases: for example, the isobases near the entrance to Cheaspeake Bay might reverse themselves and thus support Daly's (*14*) original concept of a collapsing marginal or peripheral bulge analogous to the subcrustal flow suggested by Emery and Garrison.

WALTER S. NEWMAN
STANLEY MARCH
*Department of Geology, Queens College of the City University of New York, Flushing 11367*

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## Prevention of Radiation-Induced Creatinuria by Insulin

Abstract. *Insulin completely suppressed creatinuria in rats x-irradiated with an absorbed dose of 500 rads. The hormone may exert this effect either by restoring or maintaining the ability of irradiated muscle to take up creatine from the extracellular fluid at a normal rate, or possibly by influencing the synthesis or breakdown of phosphorylcreatine.*

Previous studies have shown that animals exposed to x-irradiation exhibit a profound creatinuria (*1*). The creatinuria is dose dependent (*2*) and is maximal between the 2nd and 4th days after a single whole-body exposure. According to the current concept of creatine metabolism, radiation-induced creatinuria may be caused by any one or a combination of the following conditions: (i) increased release of creatine from damaged muscle or other organs; (ii) impaired uptake of creatine by muscle; (iii) increased rate of creatine synthesis; (iv) decreased rate of conversion of creatine to phosphorylcreatine or creatinine or both; and (v) impaired kidney function. The recent work of Gerber *et al.* (*3*, *4*) indicates that radiation-induced creatinuria results, at least in part, from a failure of muscle to utilize newly synthesized creatine at a normal rate. Furthermore, it appears that the defect in the ability of skeletal muscle to take up creatine from the blood is in part a result of direct damage to muscle caused by the incident radiation and is partially an indirect effect of x-irradiation (*5*). Although the possibility that radiation-induced creatinuria is mediated by irradiation of the adrenal (*6*) or the thyroid-parathyroid glands (*7*) appears to be excluded, it is not known if other endocrine glands are involved.

Conn (*8*) has reported recently that insulin, added to an in vitro system, enhanced the activity of highly purified creatine phosphokinase. This observation, as well as other observations made over 20 years ago, namely, that insulin promotes phosphorylcreatine synthesis in normal (*9*) and alloxan-diabetic animals (*10*), suggests that this hormone may play a role in the regulation of creatine metabolism. These considerations prompted us to study the effect of insulin on radiation-induced creatinuria, a condition which reflects a gross derangement of normal creatine metabolism.

Male rats of the Wistar strain, weighing 135 to 145 g, were caged individually in metabolism cages. The rats fed freely on Purina Laboratory Chow and were given water to drink. After a 2- to 3-day equilibration period, urine was collected daily, filtered, and then was frozen and stored at −15°C. On the 4th day, one group of rats was x-irradiated with a whole-body absorbed dose of 500 rads. The rats were irradiated in a lucite wheel (*11*) radially divided into 16 individual compartments, and were rotated under the x-ray beam at a rate of 3 rev/min. The source of radiation was a medical x-ray therapy machine operated at 250 kv (peak), 15 ma, 159 cm source to skin distance, with an added filtration of 0.5 mm Cu + 1 mm Al (half-value layer: 1.38 mm Cu). The resultant dose rate was 8.83 rad/min. Immediately after exposure and daily thereafter, the rats were injected subcutaneously with either 0.1 ml of saline solution containing 2 units of NPH insulin or with saline solution alone. Another group of rats served as controls and were injected with insulin, but were not x-irradiated. Daily urine specimens were collected for 3 days before and for 3 days after exposure of the rats to x-rays. Urinary creatine was determined essentially by Kibrick's modification (*12*) of the coupled enzyme method described by Tanzer *et al.* (*13*).

The results are shown on Table 1.

The daily creatine excretion, 0.2 to 0.3 mg/100 g of body weight per 24 hours, was not significantly different among the three groups of rats before exposure to x-rays and/or treatment with insulin, and is consistent with published data (*14, 15*). Beginning 1 day after exposure, the untreated, x-irradiated rats exhibited creatinuria which increased to maximum levels on the 2nd day and then declined on the 3rd day after exposure. Similar results have been described previously in rats exposed to ionizing radiation (*2*). On the other hand, x-irradiated rats treated with insulin excreted creatine at levels which were not significantly different from those found prior to exposure. This dose of insulin did not cause a significant change in the normal daily creatine excretion in rats which were not exposed to x-irradiation.

Table 1. Urinary creatine excretion in rats (milligrams of creatine per 24 hours). Values are means ± SE. $N$ = number of rats. $P$ is the probability, computed by comparing, within the same group of rats, the mean obtained after combining the data for the three control days, with values obtained after treatment with insulin and/or exposure to x-rays. NS, not significant.

| Treatment | Days before x-irradiation | | | Days after x-irradiation | | |
|---|---|---|---|---|---|---|
| | 3 | 2 | 1 | 1 | 2 | 3 |
| X-irradiation (500 rads) ($N = 4$) | 0.43 ± .05 | 0.27 ± .06 | 0.41 ± .05 | 1.98 ± 0.33 $P < 0.001$ | 3.10 ± 0.40 $P < 0.001$ | 2.39 ± 0.46 $P < 0.001$ |
| X-irradiation (500 rads) + 2 units insulin ($N = 6$) | 0.36 ± .03 | 0.24 ± .04 | 0.34 ± .04 | 0.28 ± .04 NS | 0.30 ± .08 NS | 0.22 ± .03 NS |
| 2 units of insulin ($N = 6$) | 0.43 ± .04 | 0.35 ± .04 | 0.31 ± .03 | 0.34 ± .05 NS | 0.45 ± .07 NS | 0.42 ± .08 NS |

In one experiment, urine from x-irradiated, insulin-treated rats was mixed with urine obtained from untreated, x-irradiated rats, and the mixture as well as each component urine specimen was assayed for creatine. The creatine concentration of the mixture was identical with the expected value calculated from the creatine concentration of each component. This finding indicated that the urine obtained from x-irradiated, insulin-treated rats did not contain substances which interfered with the enzymatic determination of creatine.

The reason for the prevention of creatinuria in x-irradiated rats by insulin is not known. If radiation-induced creatinuria is the result of an impaired uptake of creatine by muscle as suggested by Gerber *et al.* (*3, 4*), then insulin could, perhaps, relieve the creatinuria by restoring the normal uptake of creatine by muscle. In support of this view, it has been reported (*16*) that insulin was able to double the amount of creatine resorption in a preparation of surviving cardiac muscle, in vitro.

Another possible explanation for the prevention of radiation-induced creatinuria is related to the view that the hormone may influence the conversion of creatine to phosphorylcreatine in muscle. Kumta *et al.* (*17*) have reported that muscle phosphorylcreatine is decreased substantially in rats 1 to 2 days after exposure to x-rays when creatinuria is maximal. These workers suggest that the low levels of phosphorylcreatine in muscle of x-irradiated rats were probably due to an impaired phosphorylation of muscle creatine, or an accelerated breakdown of phosphorylcreatine, in either case leading to an increased excretion of creatine. It is possible that insulin promotes the uptake of creatine by muscle in x-irradiated rats by restoring the normal rate of phosphorylcreatine synthesis or breakdown, thereby preventing the blood creatine concentration from exceeding levels which would cause creatinuria. The observations made in the 1940's that insulin promoted the uptake of $^{32}P$ into phosphorylcreatine of muscle in normal animals (*9*) and restored the ability of alloxan-diabetic rats to synthesize phosphorylcreatine at a normal rate (*10*) add strength to the argument that insulin prevents radiation-induced creatinuria by its action on the creatine-phosphorylcreatine system in muscle. Nevertheless, it is also possible that the prevention of creatinuria by insulin is not necessarily related to an effect of the hormone on the phosphorylcreatine system, but on adenosine triphosphate generation (*18*).

Doubtless there are other mechanisms which can be formulated to explain the action of insulin on creatine metabolism in x-irradiated animals. Since insulin apparently can act as a radioprotective agent (*19*), the prevention of creatinuria by the hormone could be related to its radioprotective effect operating through mechanisms which are similar to, or different from, those already alluded to. Whatever the cause of the suppression of radiation-induced creatinuria, our observations have clearly demonstrated that insulin exerts a significant effect on creatine metabolism in the x-irradiated rat. However, the minimal dose of insulin which can suppress creatinuria in x-irradiated rats has not yet been established. If it is found that a dose of insulin in the physiological range will prevent radiation-induced creatinuria, then the possibility must be considered that creatinuria is influenced by the level of endogenous insulin present in the blood and tissues of x-irradiated animals. Further studies, on the relationship between insulin levels in the blood and the onset of creatinuria in x-irradiated rats, are needed. To what extent, if any, insulin can suppress or prevent creatinuria in other pathological conditions, for example, starvation, tissue atrophy, muscular dystrophy, and so forth, remains to be established.

THOMAS R. KOSZALKA
CAROLE L. ANDREW
*Departments of Radiology* and *Biochemistry, Jefferson Medical College, Philadelphia, Pennsylvania*

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