These results indirectly confirm the significance of the ATP, demonstrated by CARTIER[12], and of the cocarboxylase, demonstrated by ZAMBOTTI[1], for a normal behaviour in the osteogenic process.

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Riassunto

Studiando il meccanismo biochimico con cui la insulina controlla il processo di osteogenesi, si è stabilito che in condizioni di deficit insulinico (diabete allossanico) esiste nel ratto accanto ad un arresto dell'accrescimento scheletrico, una notevole riduzione dell'attività cocarbossilasica nella cartilagine epifisaria. Si conclude che nella cartilagine di coniugazione ATP e cocarbossilasi sono strettamente interdipendenti per quanto riguarda la loro biosintesi; inoltre che il loro normale metabolismo è indispensabile al normale svolgersi del processo di osteogenesi.

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Influence of Post Irradiation Administration of Methionine on the Excretion of Creatine, Creatinine and N-Methyl Nicotinamide in Urine of Rats

Earlier observations from this laboratory have demonstrated a precipitous fall in the levels of free-methionine and choline in rat livers[1] and an enhanced excretion of methylated end-products such as creatine, creatinine and N'-methyl-nicotinamide in urine of rats given 600 *r* total body X-ray irradiation[2]. This implied that radiation exposure probably disrupted the methyl economy and suggested that there may be a cause-and-effect relationship between radiolability of methionine[3] and the levels of the metabolites studied. These considerations prompted us to study how far replacement therapy of methionine would have beneficial effects in modifying the reported biochemical lesions of radiation exposures. In this communication, the influence of post-irradiation administration of methionine on the excretion of creatine, creatinine, N'-methyl-nicotinamide and nitrogen in urine is reported. The influence of giving methionine half an hour before irradiation has also been investigated.

Experimental.—1½ to 2 month old wister rats, inbred in our colony, were used in this investigation. The animals were divided into 5 groups of 6 rats each, as follows. The first group served as controls and the animals did not receive any treatment at all. The second group of animals were not irradiated but received injection of methionine at a dose rate of 5 mg/kg of body weight. The animals in the 3rd group were irradiated with 600 *r* X-rays. In the 4th group, animals were irradiated and received methionine ½ h after irradiation. The last group was also irradiated but received methionine before irradiation.

All animals, control as well as irradiated, were fasted for 24 h during the collection of urine. Drinking water was, however, made available to them *ad libitum*. Animals were housed in metabolic cages in pairs to facilitate the collection of urine.

For the estimation of creatine and creatinine, the urine samples were first treated with iodine-iodide reagent as described by TAUSSKY[4] to remove the interfering substances, followed by extraction of the excess of iodine with chloroform. Urine samples were then adjusted to pH 2·5 and taken for the estimation of creatine and creatinine. The conversion of creatine to creatinine was carried out by autoclaving the urine samples (pH 2·5) as recommended by CLARK *et al.*[5]. Preformed creatine and total creatinine was then estimated colorimetrically[4] by adding 1 ml of 0·04 *N* picric acid and 1 ml of 0·75 *N* NaOH. After 20 min of stabilization, the colour intensity was measured in Klett Summerson colorimeter with green filter.

For the estimation of N'-methyl-nicotinamide, the urine samples were treated with acetone and 6 *N* NaOH, followed by addition of 6 *N* HCl to produce stable fluorescent condensation product as described in detail by HUFF and PERLZWEIG[6]. The fluorescence was measured in Pfattz and Baur instrument with the combination of green and yellow filters.

Nitrogen in urine samples was estimated colorimetrically by nesslerization procedure as outlined by UMBREIT *et al.*[7].

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Influence of administration of methionine (5 mg/kg of body weight) on the excretion levels of creatine, creatinine, N'-methyl nicotinamide and nitrogen in irradiated animals.

No. of rats	Group	Nitrogen mg/day/rat	Creatine mg/day/rat	Creatinine mg/day/rat	N'MeNA mg/day/rat
4	Control	56·5 ± 0·5	0·54 ± 0·089	1·96 ± 0·28	0·236 ± 0·004
6	Control + Methionine	52·6 ± 1·52	0·57 ± 0·07	1·8 ± 0·037	0·198 ± 0·003
6	*Irradiated*	92·8 ± 4·3	1·19 ± 0·11	3·87 ± 0·24	0·425 ± 0·009
6	*Irradiated* + Methionine given *before* irradiation	85·63 ± 4·8	1·3 ± 0·2	3·42 ± 0·38	0·215 ± 0·005
6	*Irradiated* + Methionine given *after* irradiation	85·8 ± 3·39	0·463 ± 0·001	3·08 ± 0·12	0·212 ± 0·006

Standard errors have been calculated using the formula $(\Sigma d^2/n(n\text{-}1)^{\frac{1}{2}}$.

Results and Discussion.—The results of these experiments are presented in the Table. It will be seen that administration of methionine half an hour after irradiation brought back the excretion levels of creatine and N'-methyl-nicotinamide to normality, but it does not appear to have any effect on the excretion of creatinine. This continued to be high as in the case of untreated irradiated animals.

Administration of methionine half an hour before irradiation does not seem to control the increased excretion of creatine and creatinine. The only effect it had was to restore the N'MeNA levels to normality. Similarly, the excretion of nitrogen continued to be high in both the groups, thereby showing that the increased catabolic activity is not reversed by methionine.

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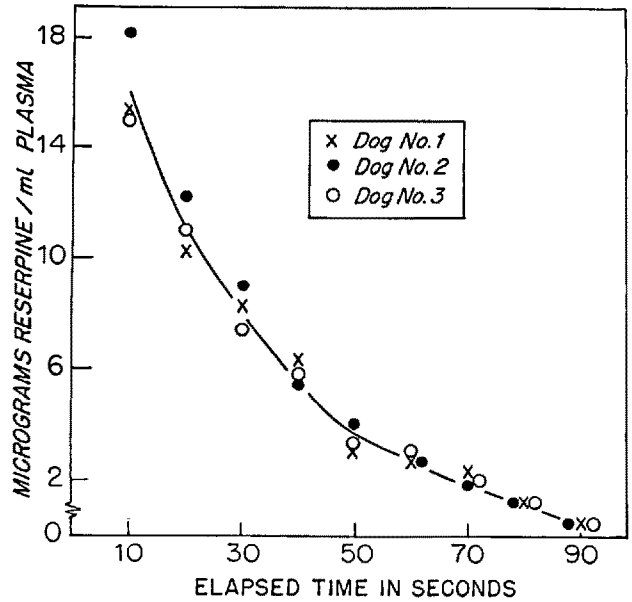
Zusammenfassung

Die Möglichkeit wurde geprüft, durch Zugabe von Methionin die Kreatin-, Kreatinin-, N'-Methylnicotinamid- und Stickstoffmengen nach Bestrahlung wieder herzustellen. Bei Methioninzugabe eine halbe Stunde nach Bestrahlung kehrte die Kreatin- und N-Methylnicotinamidmenge im Urin zur Norm zurück; wurde aber Methionin vor der Bestrahlung verabreicht, so blieben die Ausscheidungsmengen von Kreatin, Kreatinin und Stickstoff hoch.

Plasma Disappearance and Urinary Excretion of Reserpine (Serpasil®) in the Unanesthetized Dog*

Recent studies in the rat[1] and rabbit[2] indicate that the sedative effect of reserpine may not be related in time to the concentration of the drug in arterial plasma, but rather, to changes in brain 5-hydroxytryptamine concentration. Since *in vitro* and *in vivo* observations[3] suggest that there is a distinct species difference in the way reserpine is metabolized, the distribution of reserpine in arterial plasma of the dog, as a function of time, was investigated in an attempt to explain the particular sensitivity of this animal to small doses of the compound. The study was also to serve as a preliminary model for investigation of the renal handling of the drug.

A microcolorimetric method, based upon a general reaction for organic bases[4], was developed and utilized for the analysis of reserpine[5]. 6 experiments were performed on 3 unanesthetized female mongrel dogs weighing between 12·0 and 14·3 kg. They were trained and allowed free access to Purina Dog Chow and tap water. 25 ml of tap water per kg of body weight were given by mouth through a stomach tube one-half hour prior to the beginning of each experiment to insure adequate hydration and urine flow. Each animal was loosely restrained to a dog board in the supine position and an indwelling catheter placed in the bladder. The femoral artery was exposed under local anesthesia (distilled water) and a polyethylene catheter inserted into the proximal end. Control samples of urine and blood were taken. 15 min were allowed for surgical recovery. Reserpine, 1·5 mg/kg body weight, was then injected into the left external jugular vein over a 3 s interval, and arterial blood samples were taken at 10 s intervals for the next 2 min. The midpoint of the injection time was designated as zero time. Catheterized urine samples were collected at 30 and 60 min, and 24 and 48 h samples were cage-collected.



Arterial plasma disappearance of reserpine in the unanesthetized dog. The points plotted for each dog represent the averages of two experiments, and the resultant curve is a visual approximation. The broken line on the ordinate indicates the lower limit of sensitivity of the analytical method.

The disappearance of reserpine from arterial plasma is shown in the Figure. After intravenous administration, there was a prompt appearance of reserpine in arterial plasma with an average peak concentration of 16 μg/ml occurring during the first 10 s sample. Reserpine concentration then rapidly diminished to levels of less than 1·5 μg/ml of arterial plasma within 90 s.

Recovery of reserpine from urine is shown in the Table. Less than 2% of an injected dose of reserpine was recovered from urine within 24 h, and negligible amounts were found in the course of the next 48 h.

Sedation became evident at approximately 2–3 h after drug injection and persisted for 24–28 h. Tarry stools (markedly guaiac positive) were observed in all animals 3–6 h after injection. Stools were negative for occult blood within 24–48 h after injection. All dogs recovered fully in the course of one week.

The findings of a rapid disappearance from arterial plasma and low urinary excretion of reserpine are in general accord with observations of SHEPPARD *et al.*[1] in the rat using radioactive tracer doses of reserpine. HESS *et al.*[2], using 5 mg/kg of body weight in the rabbit,

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