experimental conditions.

Thymidine, as a precursor of DNA, is exclusively incorporated into the nucleus of cells synthesizing DNA prior to mitosis(2). At least 50% of the injected thymidine is incorporated into new DNA of proliferating cells, while the remainder is promptly catabolized to tritiated water and other non-volatile compounds(3). The biological half-life of the tritiated water in mice is from 24 to 36 hours(4), while the thymidine incorporated into DNA remains there until the death of the cell or its descendants. Because of the short range of the tritium beta particles (in tissues, an average of 1 μ and a maximum of 6 μ) 90% of the energy of the beta particles from H^3-thymidine is dissipated within the nucleus. On the other hand, beta particles from C^{14} have a longer range (in tissues, an average of 10 μ and a maximum of 60 μ) and most of their energy will be dissipated within the volume of a cell. Unless the carcinogenic action of radioactive thymidine is ascribed to one of its catabolites that are eliminated from the body in a few days, it would seem that radiation dissipated within the volume of a cell and perhaps even in the nucleus of a cell is sufficient to induce tumors.

Summary. Thymidine labeled with tritium or with carbon-14, when incorporated into DNA of proliferating cells, produces a selective mode of irradiation of cells and cell nuclei. Single and multiple injections of radioactive thymidine have been found to shorten the life span and to produce an increased incidence of tumors in CaF1 mice. The incidence of tumors increased with dose and it was greater in animals injected as young adults than in animals injected later in life. Labeling of embryos *in utero* with tritiated thymidine likewise increased the incidence of malignant tumors.

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A Preliminary Investigation of the Source of the Creatinuria Following Transection of the Spinal Cord. (27619)

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In our studies on the metabolic changes following spinal cord transection in dogs, it was observed that creatine was excreted in large amounts for periods of from 2 to 3 weeks after the cervical cord had been transected(1). This was in marked contrast to the slight creatinuria which occurred for a short period only in animals immobilized by removal of femurs and humeri or by cutting muscle tendons. Creatinine excretion was not altered immediately following cord transection, but fell slowly over a period of a month as the muscles affected by the cord transection atrophied. The high creatinuria set in before creatinine excretion showed any reduction. One of the many questions raised in connection with these studies was whether the creatine in the urine came from the muscles affected by the cord transection or whether it was newly synthesized creatine which the affected muscles could no longer use. To answer this question, labeled creatine was fed to a dog a few days before cervical cord transection and excretion of creatine and creatinine followed over a period of 6 weeks. Since the classical studies of Bloch

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and Schoenheimer(2) have established for creatine the rather unique absence of multiple metabolic channels, this approach to the problem seemed reasonable. The findings reported here are based on the study of but a single animal, but they seem sufficiently valid to be put on record.

Materials and methods. Preparation of N^{15}-creatine. Synthesis of N^{15}-creatine with the isotope in the glycine moiety was begun by preparing N^{15}-sarcosine from N^{15}-glycine by the methods of Fischer and Bergmann(3) with minor modernizations. The first step was toluenesulfonylation of glycine labeled with 62 atoms % excess N^{15}. The toluenesulfonylglycine resulting was methylated in alkali with methyl iodide to yield toluenesulfonylsarcosine. Heating the product in a sealed tube at 100° with hydrochloric acid released N^{15}-sarcosine as the hydrochloride. After isolation of this compound, it was condensed with methylisothiocarbamide as described by King(4) to give N^{15}-creatine which separated out as the monohydrate.

Animal experiment. The technic of cord transection, care of spinal animals, and procedures necessary for metabolic studies on such animals, have been described(1,5). An adult female dog weighing 19 lb was used. The animal had been kept in a metabolism cage and fed an egg-milk diet of constant daily amount for several months before the experiment was started. Starting 6 days before the cord transection and on 4 successive days, N^{15}-creatine hydrate, 195 mg per day, was added to the standard diet of milk and egg. Two days after the last dose of labeled creatine, the spinal cord was transected at the level of the 7th cervical vertebra. On the day of operation and on the first postoperative day, the animal was fed by intravenous infusion, a solution containing amino acid (Aminosol, Abbott), glucose, K_2HPO_4, and NaCl, adjusted to keep the intake of nitrogen, sodium, and potassium the same as when the regular diet was fed. After the first postoperative day, the standard diet of milk and egg was resumed, but it was administered by stomach tube. Urine was collected through an indwelling catheter in the bladder, maintained for 6 weeks postoperatively. The 24-hour urine samples were frozen immediately after collection.

Biochemical methods. Urinary creatine, creatinine, and total nitrogen and food nitrogen were determined as described previously (5). Daily measurements of urinary nitrogen, creatine and creatinine were made. Nitrogen balance was estimated on the basis of fecal nitrogen being considered 10% of that ingested.

For measurement of the N^{15}-labeled material, the creatine and creatinine fractions were isolated from the urine in the form of K creatinine picrate by a method essentially the same as that described by Benedict *et al.*(6). After benzene extraction of picric acid from these products in acid aqueous solution, refluxing in 10% barium hydroxide, and removal of barium, the sarcosine released was isolated as the toluenesulfonyl derivative. The nitrogen of this product was separated as ammonium sulfate by the Kjeldahl procedure, and prepared for determination of its N^{15} abundance in the mass spectrometer.

Results. In the first 48 hours following the cord transection at C_7, there was a profound depression of the animal's activity involving the entire body. After a few days, the muscles innervated by the segments of the cord above the transection resumed their normal muscle tone and activity. All ordinary activity was abolished in that part of the body innervated below the transection and the muscles were flaccid, but contraction of these flaccid muscles could be stimulated by tapping on tendons. Handling of the animal such as catheterizing the bladder was sufficient stimulation to cause active kicking and trunk movements and wagging of the tail. Body weight fell from 9 kg to 7.8 kg in the 6-week postoperative period and the nitrogen balance showed a loss of about 1 g N a day.

Urinary excretion of creatine and creatinine is shown in Fig. 1. Urinary creatine was high for 5 days postoperatively, but in the next 8 days only a negligible amount was excreted. The creatine excretion then rose again and remained elevated for about 20 days. The absence of creatinuria for 8 days contrasts with the usual, but not invariable, continuous creatinuria which follows cervical

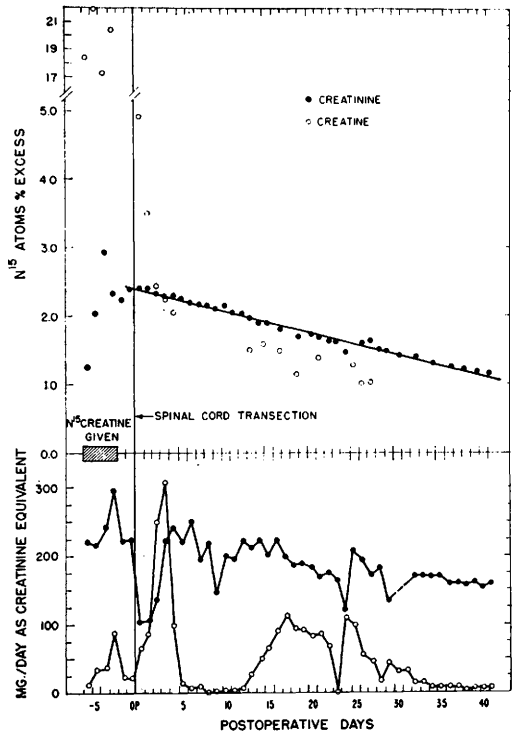

FIG. 1. In the lower section, the amounts of urinary creatine (○) and creatinine (●) excreted daily are plotted as equivalent mg of creatinine against time in days related to day of spinal cord transection (vertical line at 0). In the upper section, the atoms % excess of N^{15} found in the creatine and creatinine isolated for the day are plotted similarly against time.

cord transection and lasts through the 10th-20th postoperative day. Creatinine excretion, after an initial drop which is perhaps related to temporarily disturbed renal function, gradually fell over a period of one month to a level about two-thirds of its preoperative value. This latter course is characteristic of the creatinine excretion pattern after spinal cord transection in dogs and seems to occur parallel with the muscle wasting.

In the upper part of the chart are plotted the values for the N^{15}-abundance in the glycine nitrogen of the isolated creatine and creatinine. Values for the N^{15}-creatinine fraction fell gradually during the period of observation. Variation from a straight line may be explained, in part, by difficulties in handling the small quantities of creatine and creatinine present in daily excretions. N^{15} values obtained for the very small amounts of creatine excreted during the preoperative period are scarcely more than qualitatively significant, because of certain errors, such as correction for a low baseline creatinuria and dilution by addition of carrier creatine, creatinine, or ammonia at various stages during isolation of the small quantities obtained. The time period allowed between N^{15}-creatine ingestion and cord transection appears to have been too brief for complete absorption and assimilation of the labeled creatine to occur before the operation, since the N^{15}-abundance of the creatine excreted during days 1 and 2 postoperatively continued to be high. From the 14th to the 28th postoperative days, creatine reappeared in the urine. Its degree of labeling closely paralleled that of the urinary creatinine, though it was consistently 15-20% lower than that of the creatinine excreted on the same day.

Discussion. Our purpose was to identify the origin of the excessive urinary creatine appearing after cord transection, *i.e.*, to ascertain whether the creatinuria was due to "leakage" of creatine from muscle, presumably from those muscles affected by the cord transection, or to an excess of creatine in the body because of the inability of the flaccid muscles to take up newly synthesized creatine. Bloch and Schoenheimer(2) showed, in experiments in which body tissue creatine had been labeled with N^{15}-creatine, that body creatine, which is mainly muscle creatine, is the source of the urinary creatinine. They showed that the creatinine carried the same degree of N^{15}-labeling as muscle creatine. This identifies the labeled muscle creatine with the urinary creatinine. Since the N^{15}-abundance of the creatine excreted during the periods of day 14 to day 28 was only a little less than that of the N^{15}-abundance of the creatinine excreted for the same day, the conclusion seems warranted that most of the creatine excreted during this period comes from muscle. If the urinary creatine had been the creatine synthesized concurrently but left unincorporated because the muscles were no longer able to "ingest" their needed quota of the compound, it would not have been labeled and thus no N^{15}-labeled creatine would have been found in the urine. The consistent difference

between the N^{15}-abundance in the creatinine and creatine, with the creatine about 15-20% less, suggests a dilution of the creatine which has come from muscle with creatine which had been currently synthesized but which was not taken up by the altered muscles.

The value for the half-life of body creatine may be determined from the abundance of N^{15} in the urinary creatinine excreted over a period of time. From the graph, it can be seen that 38 days were required for the creatinine N^{15} to drop from 2.4 atoms % excess at time of operation to half that value, or 1.2 atoms % excess. In the intact human subject studied by Hoberman, Sims, and Peters(7), this half-life was 42 days; Bloch, Schoenheimer, and Rittenberg(8) found the half-life of creatine in the intact rat to be 29; and Cohn, *et al.*(9) found it to be 36 days. The daily decrease in abundance of urinary creatinine N^{15} may be estimated to approximate 2%. This represents the proportion of body creatine leaving the tissues daily after "spontaneous" transformation to creatinine. Even in the presence of much greatly disordered muscle tissue, it is similar to the corresponding values (1.9-2.1%) found for the rat by Bloch, Schoenheimer and Rittenberg (8), and for man (1.64%) by Hoberman, Sims, and Peters(7).

These findings with regard to the origin of the urinary creatine following spinal cord transection are in contrast with the findings for the origin of the urinary creatine in progressive muscular dystrophy as shown by Roche *et al.*(10) and Benedict, *et al.*(6). These authors showed that the creatine excreted by their patients with muscular dystrophy was newly synthesized creatine which could not be utilized by the diseased muscle.

Accompanying the loss of tone and inactivity of all the muscles innervated by that part of the cord which was caudal to the transection in this dog, there was severe atrophy of these muscles. This kind of muscle atrophy has been characterized as atrophy of disuse by Tower(11,12) who studied muscle innervated by a segment of isolated spinal cord in puppies and found that the root ganglia and peripheral nerves of this segment are composed in large part by healthy cells and nerve fibers, but the skeletal muscles innervated by the isolated spinal cord are atrophic; the muscle fibers are reduced in all dimensions, are pale staining and some of the fibers are in the process of transformation into fibrous tissue. Interstitial fibrous tissue is also increased in the muscle bundle. In view of this striking dissolution of those muscle fibers affected by the cord transection, it is to be expected that changes in muscle chemistry and in permeability are taking place which are associated with "leakage" of creatine and other constituents from the muscle fibers.

Summary. It has been shown that the creatine excreted in large amount following spinal cord transection originates mainly from the muscle tissue which has been affected by the cord transection. Creatine half-life in the "spinal" dog was 38 days. The rate of disappearance of body creatine in the dog (estimated to be about 2% per day) is compatible with that found for other species.

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Changes in Corticosterone Levels after Severe Stress in the Rat. (27620)

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Many measurements of free corticosteroids after various forms of stress have been noted in the literature(1). Rennels and Timmer (2) noted that free plasma corticosterone levels in the rat reached a peak level about one hour after scalding and remained elevated for 24 hours. Knigge *et al.*(3), using similar stress and adrenal vein cannulation technics, reported normal values 4 hours after scalding and a marked decrease in corticosterone secretion 12-36 hours after scalding. Rennels and Timmer(2) found maximum hemoconcentration as measured by hematocrit values occurred at one to 3 hours after stress. Marked reduction in blood volume following burns, which is due mainly to increased capillary permeability with resultant plasma loss, has been well documented(4). The extensive plasma loss occurring after burns raised the question as to how accurately the concentration of corticosterone in the blood plasma actually reflects adrenal corticosteroid output. For these reasons the author collected "burn tissue fluid" and measured its concentration of free corticosterone. The substance measured in burn tissue fluid was presumed to be corticosterone on the basis of the work by Silber, *et al.*(5) and will be referred to as such although its identity has not been rigidly established in burn tissue fluid. Previous reports have indicated that after periods longer than 24 hours hematocrit values returned toward normal levels accompanied by a marked reduction in edematous fluid volume(4). In the present study measurements were made of hematocrit values, free plasma corticosterone levels and free corticosterone in "burn tissue fluid" at time periods up to 168 hours after the burn stress.

Methods. Male Sprague-Dawley rats, weighing approximately 230 g were anesthetized with sodium pentobarbital (4.5 mg/100 g body weight) and immersed except for the head in 70°C water for 5 seconds. Animals were decapitated at one-half, 3, 12, 24, 48, 72, 96, 120 and 168 hours after burning. The blood of each animal was collected in heparinized tubes, immediately centrifuged, and determinations of free plasma corticosterone were made using the method of Guillemin *et al.*(6) with specificity of corticosterone established by Silber *et al.*(5) and Peterson (7). Hematocrits of individual animals were taken at time of decapitation. "Burn tissue fluid" was collected from the edematous feet of burned animals after decapitation by applying pressure to these areas and collecting the fluid in a heparinized tube. "Burn tissue fluid" was defined as all fluid obtained by applying pressure to the edematous feet, recognizing that most fluid obtained was extracellular, with possibly some intracellular fluid and small amounts of blood. Appropriate control animals were maintained for all groups.

Results. The highest level of free plasma corticosterone (52.5 μg/100 ml of plasma) was found in burned animals 3 hours after stress (Table I). All groups killed at time periods greater than 3 hours showed a progressive decline in concentration of plasma corticosterone. Approximately 48 hours after stress the animals had returned to resting levels (12 μg/100 ml plasma) of free plasma corticosterone. With one exception (72 hours) only slight variations from this level were found during the next 120 hours. Hematocrit values of burned animals showed a peak