Effect of Thermal Injury on Ascorbic Acid and Tyrosine Metabolism. (26305)

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(Introduced by R. W. Clarke)
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It is well established that stress alters many metabolic patterns of the animal organism extensively(1). The metabolism of Vit. C has been studied in this context, and it appears that a severe injury imposes a need for Vit. C far above normal. This conclusion is based on studies of Vit. C tissue saturation and blood levels, and wound healing(2). The present investigation was aimed at determining whether the increased need for Vit. C after stress could be demonstrated for an enzymatic function in which this vitamin is known to be involved, namely, metabolism of tyrosine.

Garrod(3) made a significant contribution to the theories of the metabolism of the aromatic amino acids by correctly ascribing alcaptonuria to an inborn error of metabolism. Alcaptonuria is marked by appearance in the urine of homogentisic acid (2,5 dihydroxyphenylacetic acid) and is caused by a lack or absence of the enzyme necessary to carry the conversion of tyrosine further. It was subsequently discovered almost simultaneously by Sealock(4) and Levine(5) that Vit. C is required for formation of homogentisic acid from its precursors. Sealock(6) also showed conclusively by differential colorimetry and by preparation of derivatives, that the urinary acids resulting from feeding l-tyrosine or l-phenylalanine to scorbutic guinea pigs are primarily p-hydroxy phenylpyruvic acid and smaller amounts of homogentisic acid; this established that the metabolic defect of scurvy was fundamentally different from inherited alcaptonuria.

The present study was designed to answer the following. (A) Does a severe burn cause appearance of abnormal urinary compounds even when the animals are provided 2 mg/day Vit. C, an amount known to be sufficient for normal growth and wound healing(2,7); (B) if so, what is the nature of the compound or compounds; and (C) can this be prevented by administration of larger doses of ascorbic acid?

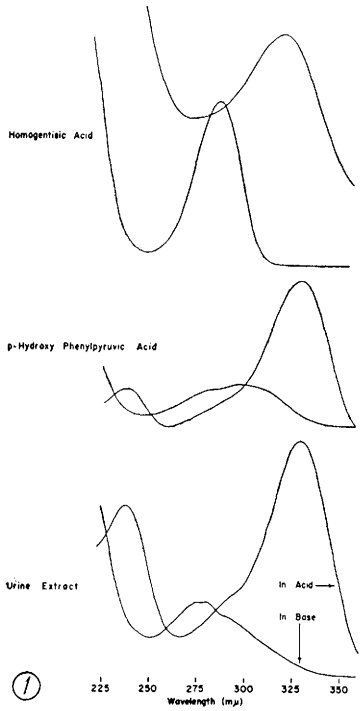
Materials and methods. Male guinea pigs (200 ± 10 g) were used, obtained from Fort Detrick, Md. (Hartley strain). They were offered, *ad libitum,* a ground Rockland* diet that was nutritionally complete except for a total lack of Vit. C. The diet was supplemented (except for those animals which were meant to become Vit. C deficient) with 2 mg ascorbic acid in 0.5 ml water administered each day by pharyngeal tube.

The guinea pigs were housed in metabolic cages in an air conditioned room, the temperature of which was kept at 78° ± 1°F. The cages were provided with plastic funnels, with glass urine-feces separators attached. Each day, cages and accessories were cleaned and the water changed.

The tyrosine load test was performed as follows: 0.75 mg l-tyrosine per gram of body weight was administered by stomach tube as a slurry in approximately 10 ml of 0.9% saline.

Urine was collected on a 24-hour basis. Each collector contained 5 ml of 2 N hydrochloric acid, an amount sufficient to maintain the urine acid to litmus at all times. Each collection was filtered to remove solid particles, the filter paper washed twice with water, and the filtrate was diluted to 100 ml. An aliquot of one-tenth of this volume was extracted 3 times into ethyl ether. This step excluded all inorganic compounds and organic basic compounds. The ether phase was reextracted into 5% sodium bicarbonate solution. This step excluded weak acids, such as phenols. Finally the sodium bicarbonate solution was acidified with hydrochloric acid and extracted into ether. The ether was

* Obtained from the A. E. Staley Mfg. Co., Decatur, Ill.



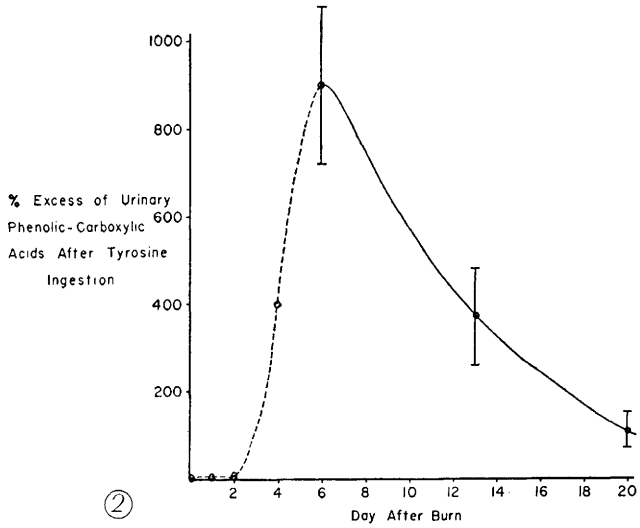


FIG. 1. Ultraviolet spectra of urine extract of burned guinea pig, p-hydroxy phenylpyruvic acid, and homogentisic acid, all in acidic and basic water solutions.

FIG. 2. Effect of burn on tyrosine metabolism: Percent excess of phenolic-carboxylic acids in 24 hr urine after tyrosine ingestion (0.75 mg/kg body wt) compared with 24 hr urine before tyrosine ingestion. All guinea pigs were maintained on 2 mg/day vit C. Dotted line denotes single guinea pigs burned at 73°C; solid line, 19 animals burned at 85°C. Vertical lines on the curve denote stand. error.

evaporated to dryness and the residue taken up in 25 ml absolute ethanol. This procedure could be expected to isolate strongly acidic organic substances, including carboxylic acids and carboxylic phenols. The alcoholic solutions were then examined spectrophotometrically at 297 mμ, using a Beckman DU instrument. Spectra of pure compounds and, in some cases, urine extracts were obtained with a Cary Model 11 recording spectrophotometer.

Some animals were subjected to a standard back burn under ether anesthesia(8). Burns were produced by dipping the guinea pigs' backs into water containing a wetting agent, polyoxyethylene lauryl alcohol.† The burns were administered at 73° or 85°C, involved one-third of the body surfaces and were third degree. Half the animals were treated immediately post burn with 0.9% saline and half with 0.9% saline containing 5% dextrose. These solutions were administered by stomach tube in an amount equal to 5% of the body weight. There were no significant differences in survival rates of these groups (one of each group died), or their tyrosine tests. The tyrosine test results, therefore, have been combined for presentation.

Results and discussion. Fig. 1 shows the ultra-violet spectra of urine obtained from a burned guinea pig after tyrosine feeding, and of 2 pure compounds known to be formed *in vivo* from tyrosine *i.e.*, homogentisic acid and p-hydroxy phenylpyruvic acid. All 3 show the transposition and splitting of maxima characteristic of phenols. The urine extract spectrum seems to be a composite of the 2 compounds, with p-hydroxy phenylpyruvic acid greatly predominant. Small amounts of other acidic aromatic compounds may, of

† BRIJ 35, obtained from Atlas Powder Co., Wilmington, Del.

course, also be present. At any rate, homogentisic acid is at most a minor component. These results show that tyrosine metabolism is altered as a result of a severe burn, and that the abnormality is probably due to a derangement of Vit. C metabolism, since the abnormal urinary components of the burned guinea pig are apparently identical to those found in the urine of scorbutic guinea pigs.

Sealock and Silberstein(6) showed that fed tyrosine yielded urinary phenolic acids if Vit. C was omitted from the diet of the guinea pig for only one day. Painter and Zilva(9) confirmed this, and we also observed the same rapid onset of the abnormality in experiments preliminary to the present study.

Two groups of workers(10,11) have now demonstrated that livers of Vit. C deficient guinea pigs which had been given excess tyrosine have both an increase in activity of the enzyme system producing p-hydroxy phenylpyruvic acid and a decrease in activity of the enzyme system which oxidizes it, thus accounting for its accumulation in those animals.

In addition, McElroy and Anderson(12, 13) have shown that tourniquet injury inflicted on rats results in a 3-fold increase in ability of the isolated rat liver to produce p-hydroxy phenylpyruvic acid from tyrosine. They concluded that this striking increase in activity was due to suppression of an inhibitor to tyrosine α ketoglutarate transaminase, rather than to an increase in the transaminase itself, and that rate of reaction of tyrosine with pyridoxal phosphate (the first step in transamination), was thereby specifically increased.

These findings are consistent with those of Knox(10) and Zannoni(11) and implicate derangements of both p-hydroxy phenylpyruvic acid producing and degrading systems, whether due to ascorbic acid deficiency or to some other cause.

Fig. 2 shows results of our experiment involving 19 burned guinea pigs. The figures on the abcissa refer to the day just before administration of test dose, and the day just after. All urines were also analyzed the second and third days after the tyrosine test, but were invariably normal.

In view of the heterogeneity of the compounds we measured we have not attempted to relate our absorbency units to a specific compound, but have calculated total "optical density" units for each day's urine from the aliquot which we took for analysis. The data show conclusively that the burn creates an additional need for Vit. C beyond the 2 mg/day which are normally sufficient to maintain the enzymatic systems necessary for tyrosine metabolism. This requirement had been established by preliminary experiments. The metabolic derangement reaches its peak on or near the sixth day after the burn, and declines almost linearly thereafter. One guinea pig, not represented on this graph, was maintained on 100 mg/day Vit. C after the burn, and did not react abnormally to the tyrosine test performed on the fifth post-burn day. Further work is required to establish minimum Vit. C supplement necessary to abolish the abnormal post-burn response.

This pattern of metabolic reaction to injury as it involves Vit. C agrees with that previously observed by Levenson, *et al.*(2), who found that healing of wounds of burned guinea pigs was strikingly abnormal during the first postoperative week. These wounds were histologically indistinguishable from those of guinea pigs depleted of Vit. C by nutritional deprivation. The abnormalities were still present 10 days postoperatively, though to a lesser extent, and by the fourteenth day, the wounds were almost normal. These animals received 2 mg Vit. C per day. Addition of 100 mg/day Vit. C to the basal diet allowed the wounds to heal normally, despite the superimposed burn. The authors ascribed the abnormal wound healing to derangement of ascorbic acid metabolism induced by the burn. Our findings confirm this hypothesis.

Summary. Intragastric administration of 0.75 mg of l-tyrosine per g body weight to guinea pigs maintained on 2 mg of Vit. C per day did not yield urinary tyrosine intermediate metabolites. Burned guinea pigs to which this test was applied at various times after the burn showed an abrupt abnormal response on the third post burn day despite

a daily maintenance Vit. C dose of 2 mg per day. This response was manifested by appearance of large amounts of urinary p-hydroxy phenylpyruvic acid, the same compound which appears in the urine of uninjured guinea pigs fed tyrosine when ascorbic acid is removed from their diet. Metabolic abnormality reached its peak on the sixth post burn day, then declined to almost normal levels by the 20th post burn day. One hundred mg per day of Vit. C abolished the abnormality after the burn. These results agree with previous observations of altered Vit. C metabolism and an increased need for this vitamin after burns, as judged by the abnormal wound healing within the first 2 weeks after a burn.

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A Method for Measurement of Urinary Deoxyribonuclease Based on the Reaction of Indole with Deoxyribonucleotides.* (26306)

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The need for a more precise sensitive method to determine the activity of urinary deoxyribonucleases I and II (DNase I and DNase II) has led us to develop such a method based upon the indole reaction originally described by Dische(1) and used by Ceriotti for estimation of deoxyribonucleic acid(2). The failure to obtain good reproducibility even with zero time blanks, as noted with other urinary DNase methods(3,4), has been eliminated by insuring the quantitative precipitation of unreacted DNA. This has been accomplished by adding a protein such as casein or bovine serum albumin to the enzyme reaction mixture.

The use of purified, freshly distilled chloroform in Ceriotti's procedure has been circumvented by substituting commercially available analytical grade benzene as extracting solvent.

Insight into the mechanism of the indole reaction has been gained by comparing reactivity of purine and pyrimidine deoxyribotides and the corresponding deoxyribosides as well as that of certain possible hydrolysis products of DNA.

The method developed primarily for measurement of urinary DNase activity has been found equally applicable to measurement of DNase in serum and tissue homogenates. However, only a general method for determination of DNase I will be discussed here.

Experimental. I. *Reagents.* A. *General reagents for DNase I and II*: (a) A 0.2 g% solution of sodium deoxyribonucleate (DNA). DNA was prepared in this lab-

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