paraffin stimulated whole saliva and blood serum in 34 patients from whom samples were collected simultaneously. 1) In every patient, the buffer capacity of serum exceeded that of saliva in the pH range tested; in each, saliva contained higher concentrations of potassium and phosphate than serum, and serum contained greater concentrations of sodium, bicarbonate and protein than saliva. 2) There was no statistically significant correlation between the buffer capacities and the main buffer constituents of serum and stimulated whole saliva. 3) Thus, the quantitative composition of the salivary buffers which act to protect against dental caries is determined primarily by glandular activity and not by simple ultrafiltration.

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Role of Enzymatic Adaptation in Production of Experimental Alkaptonuria.* (23520)

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Papageorge and Lewis reported that rats maintained on high phenylalanine diets began, after a lag period of as long as 3 weeks, to excrete homogentisate in their urine(1). After this demonstration it was no longer possible to consider, as Dakin had, that homogentisate was an abnormal metabolite formed only by individuals with the rare hereditary disease of alkaptonuria. It has since been well established that homogentisate is normally formed in mammalian liver in the course of the metabolism of tyrosine and phenylalanine (2). Biochemical genetics has suggested a mechanism for hereditary alkaptonuria: the inactivity of the genetically controlled enzyme that degrades homogentisate. No mechanism has been suggested for the dietary alkaptonuria, but the lag period before its appearance suggests that some metabolic adaptation (11) must occur. In the present studies, the activities of the several enzymes acting successively to form and degrade homogentisate have been measured during the production of experimental alkaptonuria in rats. It was expected that adaptive changes in the amount of one enzyme relative to another would occur which would account for the accumulation of homogentisate. This expectation was con-

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firmed. Tyrosine feeding was used in the present experiments for economy of materials and assays, since one less enzyme step was involved in the production of homogentisate from tyrosine than from phenylalanine. It was found that alkaptonuria could be as well produced by tyrosine- as by phenylalanine-rich diets, in preliminary experiments with Drs. Auerbach and LeMay (these studies did not substantiate the development of arthritis in rats during the long continued alkaptonuria (3)). Homogentisate was measured in the urines of 27 rats fed the high tyrosine diet as long as 7 weeks. Animals were killed at intervals during this time for measurement of the levels of the appropriate liver enzymes for correlation with the degree of observed alkaptonuria.

Procedures. Animals. Male albino Slonaker rats weighing 140-250 g were used. The rats were fed, depending on their weights, 10-15 g/day of powdered Purina Rat Chow containing 5% L-tyrosine. Urines were collected over 24-hour periods in flasks containing 2 ml of 50% glacial acetic acid. *Analysis of Urines.* Homogentisic acid was determined by the iodometric titration method of Neuberger(4). *p*-Hydroxyphenylpyruvate (pHPP) was determined by converting it to the enol borate complex(5). To the urine sample was added 3 ml of 1 M boric acid in 2 M arsenate at pH 6.5. The solution was diluted to a final volume of 3.5 ml and after 5 minutes the optical density was read at 310 mμ against a blank which contained every component except boric acid. pHPP ($\epsilon_{310\,m\mu} = 1.24 \times 10^4$) was either absent or present in less than 0.1 mg in several urine samples containing homogentisic acid. Therefore pHPP was not systematically followed in these experiments. *Assay of Enzymes.* All enzyme assays were determined in fresh supernatant fractions of 10% liver homogenates. The livers were homogenized in .14 M KCl in 0.005 N NaOH. The enzyme protein was the sole limiting factor in each of the assay reactions. Activities were expressed as μmoles of substrate reacting/hour/g of dry liver. Tyrosine-α-ketoglutarate transaminase was assayed by a spectrophotometric method which depended upon

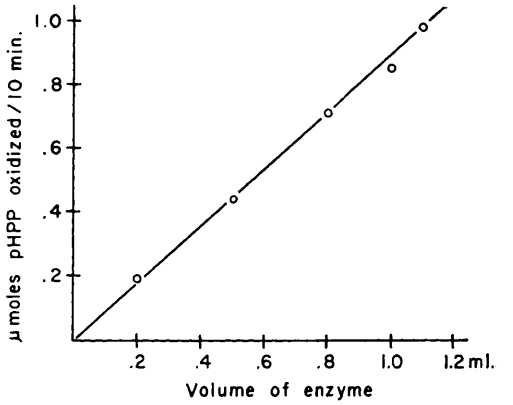

FIG. 1. Activity-concentration curve of rat liver pHPP oxidase measured by the disappearance of the enol-borate complex of pHPP. The enzyme was assayed at 25° ± 1°C in a 1 cm quartz cell containing 0.4 μmole pHPP, an excess of a preparation of kidney keto-enol tautomerase ($k = 1.0$) free of pHPP oxidase(5), 0.57 M borate, pH 8.0 in a final volume of 3.5 ml. The pHPP was allowed to equilibrate for 5 minutes in the borate buffer in the presence of the keto-enol tautomerase before addition of the oxidase. The reaction was initiated by the addition of the rat liver extract. The disappearance of pHPP ($\epsilon_{310} = 9.85 \times 10^3$) was followed for 5 minutes by readings of optical density at 310 mμ, against a blank containing all components of the reaction mixture except pHPP.

the appearance of the enol-borate complex of pHPP(6). *p*-Hydroxyphenylpyruvate oxidase was assayed by spectrophotometric method with slightly different conditions from those described by Hager, Gregerman and Knox(7). The conditions used are given in the caption for Fig. 1, which shows the proportionality of activity to the amount of enzyme used in this assay. Homogentisate oxidase was assayed by the manometric method described by Knox and Edwards(8), with the extra addition of 10 μmoles of glutathione to the reaction mixture to insure full activity. The rate of oxygen uptake was proportional to the amount of enzyme used over a 4-fold range.

Results. The majority of the rats began to excrete homogentisate 3 to 4 days after they were placed on the high tyrosine diet. The mean 24-hour excretions of homogentisate during the period of 7 weeks, constructed from 67 urine collections on 27 animals, are shown in Fig. 2. During the first month the amount of homogentisate excreted rose slowly to a peak of about 100 mg/day and

TABLE I. Correlation of Enzyme Levels in the Liver with Homogentisic Acid Excretion by Male Rats on a High Tyrosine Diet.*

Treatment	Homogentisic acid (mg in 24 hr urine)	Tyrosine transaminase	pHPP oxidase	Homogentisate oxidase
	(Mean ± S.E.)			
Purina chow diet		159 ± 21 (23)†	260 ± 25 (16)	565 ± 22 (15)
High tyrosine diet				
Group I	1 ± .48 (8)	104 ± 25 (8)	233 ± 31 (5)	411 ± 76 (8)
II	27 ± 4.5 (8)	139 ± 45 (8)	282 ± 50 (6)	400 ± 48 (8)
III	81 ± 8.9 (11)	173 ± 40 (11)	268 ± 32 (10)	358 ± 27 (11)

* Enzyme activities are expressed as μmoles of substrate converted/g dry wt of liver/hr. All assays were carried out at 25°C, except homogentisate oxidase which was measured at 37.7°C.
† No. of animals.

then declined. Fewer animals were observed in the late period, so the decline in amount may not be significant.

The rats were grouped according to the amount of homogentisate they individually excreted shortly before they were killed for enzyme assays, regardless of the length of time they were on the diet. Group I included all rats that excreted less than 5 mg of the acid in 24 hours, Group II included rats that excreted from 5-50 mg, and Group III included the rats which excreted more than 50 mg of the acid. The mean levels in control rats and in the 3 groups of experimental rats of homogentisate excreted and of the liver enzyme levels are given in Table I. No significant changes in the levels of *p*-hydroxyphenylpyruvate oxidase occurred. Rats on the experimental diet had lower activities of the homogentisate oxidase and tyrosine transaminase than those on a stock diet. No explanation of the lowered enzyme levels in these animals can be offered, although it might be mentioned that on this diet they grew like the controls and at the end of 8 weeks showed an average gain of 40% of the initial body weight. The homogentisate oxidase levels in the 3 groups on the experimental diet were not significantly different from each other. The tyrosine transaminase levels were lowest in Group I, intermediate in Group II, and highest in Group III, paralleling the amount of homogentisate excreted by these groups. Exactly the same relationship between enzyme activity and alkaptonuria was found when groups of rats sacrificed early and late in the experiment were compared.

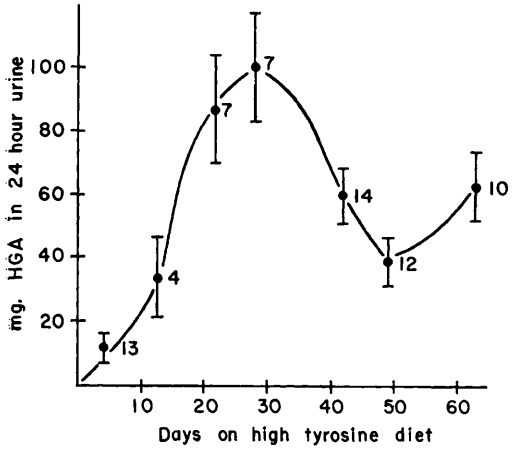

FIG. 2. Excretion of homogentisic acid by rats on high tyrosine diet. The mean homogentisic acid content in 7 groups of urine collections from male rats on a 5% L-tyrosine diet are represented by the dots. The lengths of the bars are equal to 2 × S.E. The number of urine collections at each interval is indicated by the number near each dot.

The ratio of tyrosine transaminase activity (the effective source of homogentisate) to the homogentisate oxidase activity (which disposed of homogentisate) should determine the steady state concentration of homogentisate available for urinary excretion. These ratios, calculated from the enzyme assays on individual animals and then averaged, are given in Table II for the controls and for each of the 3 groups of rats. There are highly significant differences in the ratios for the different groups, with the higher ratios, representing a surplus of homogentisate formation over disposal, being present in the groups excreting more homogentisate.

Discussion. Experimental alkaptonuria provides an excellent opportunity for the study of the relation between an altered living

TABLE II. Correlation of Ratio of Tyrosine Transaminase Activity to Homogentisate Oxidase Activity in the Liver with Homogentisic Acid Excretion.

Rats	Homogentisic acid (mg in 24 hr urine) (Mean ± S.E.)	Tyrosine transaminase / Homogentisate oxidase (Mean ± S.E.)
Controls	——	.24 ± .020 (14)
Group I	1 ± .48 (8)*	.26 ± .022 (8)
II	27 ± 4.5 (8)	.36 ± .045 (8)
III	81 ± 8.9 (11)	.49 ± .036 (11)

Statistical significance of the differences in enzyme levels between groups, as evaluated by the *t* test, is as follows: $P < .1$ for Group I *vs* Group II; $P < .05$ for Group II *vs* Group III; $P < .001$ for Group I *vs* Group III.

* No. of animals.

state and the changed enzyme levels in tissues. All the relevant enzymes occur predominantly in one organ, the liver, and they all can be measured. Any increased formation or decreased degradation of homogentisate producing a greater supply would be immediately reflected in an increased rate of renal excretion, since in man, at least, homogentisate is cleared by the kidney at the glomerular filtration rate(10). The enzymes concerned with both the formation and the degradation of a metabolite must be measured to identify the critical enzyme changes causing the accumulation of the metabolite. The occurrence of physiologically significant adaptive changes in the enzyme levels will be revealed by the appearance of alkaptonuria, and a quantitative comparison can be made between the degree of the enzyme change and the amount of homogentisate excreted. Finally, the absolute values of enzymic rates measured *in vitro* have no direct relation to the rates of the reactions which occur *in vivo*, and the identification of the limiting reactions must depend upon the correlation of enzyme changes with the metabolic changes.

The decrease in homogentisate oxidase without change in the pHPP oxidase which occurred when the rats were given the experimental diet was the sort of metabolic adaptation which might have led to alkaptonuria. That it did not (Group I) suggests that homogentisate oxidase was not the limiting reaction under these conditions. The fact that no pHPP was excreted and that the unchanging activity of pHPP-oxidase could transmit the effects of the increased activity of the transaminase preceding it in the metabolic pathway (Groups II and III) suggests that pHPP oxidase activity was also greater than that of the other enzymes under the conditions present in the cell. Tyrosine transaminase, the effective source of homogentisate in this metabolic sequence, and homogentisate oxidase, the degradative reaction for homogentisate, were present in near rate limiting amounts. This is indicated by the absence of alkaptonuria when the amounts of both these enzymes were reduced by the experimental diet, so long as the ratio of their activities remained the same as in normal animals (Group I), and by the appearance of alkaptonuria when the ratio of transamination to homogentisate degradation increased (Groups II and III).

In all known forms of alkaptonuria there is a predominance of homogentisate formation over its metabolic removal, and renal defects do not cause the condition. In the hereditary form of human alkaptonuria the discrepancy between formation and removal is referrable to decreased removal due to relative inactivity of the genetically controlled homogentisate oxidase. The chemical inhibition of this enzyme by α-α-dipyridyl *in vivo* has produced a similar form of alkaptonuria (9). In the experimental alkaptonuria studied here the predominance of homogentisate formation over its removal is referrable to increased formation due to an adaptive increase in the tyrosine transaminase, possibly the result of the high substrate feeding(6). The amount of this increase (Group I compared with Group III) represented an increase in the potential transaminating ability of a whole rat from 15 mmoles to 24 mmoles per day, equivalent to the formation of 1.5 g of extra homogentisate per day. Less than 10% of this amount would have to escape degradation by homogentisate oxidase for the observed excretion of up to 100 mg of homogentisic acid per day to occur.

Summary. Experimental alkaptonuria of rats maintained on a high tyrosine diet is associated with an increased ratio of activity of

the tyrosine transaminase to activity of the homogentisate oxidase in the liver. This type of alkaptonuria is therefore the result of an adaptive increase in relative rate of homogentisate formation, in contrast to the relative decrease in rate of homogentisate degradation believed to be responsible for hereditary alkaptonuria in man.

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Role of Potassium in Regulation of Coronary Blood Flow.* (23521)

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Katz and Lindner(1) have shown that small increments in the concentration of potassium (K) in defibrinated blood perfusing the fibrillating dog heart result in coronary vasodilation, whereas large increments of K produce vasoconstriction. Dawes(2) observed a similar relationship between blood K concentration and changes in arterial flow in the perfused hind limbs of cats and dogs. Since small increases in K levels decrease vascular resistance and since K is released from actively contracting muscle(3,4), Dawes(2) suggested that the K ion originating in the muscle may be responsible for the increased flow which accompanies muscular contraction. The present study was undertaken to determine to what extent the coronary vasodilation, which occurs during increased metabolic activity of the myocardium, could be accounted for by the release of K from muscle cells.

Methods. Ten dogs weighing 14.6 to 20.7 kg were used in these experiments. The animals were anesthetized with sodium pentobarbital (30 mg/kg) and the chest was opened in the fourth left intercostal space. Lung inflation was maintained by positive pressure artificial respiration and heparin (180 mg) was given to prevent blood clotting. The coronary sinus was cannulated via the right atrium and the venous outflow directed through rubber tubing into the right external jugular vein. The left coronary artery was cannulated with an Eckstein cannula(5) and received blood from the right common carotid artery via a pump perfusion system which maintained a constant perfusion pressure independent of aortic pressure. Mean coronary blood flow (CBF) was measured by an optically recording rotameter and mean perfusion pressure (PP) and phasic aortic pressure (AP) were recorded by modified Gregg manometers. In the first set of experiments several concentrations of KCl were infused proximal to the rotameter (to insure complete mixing of blood and infusate) at a constant rate of 0.93 cc per minute. Concentrations of KCl were used which were calculated to increase the animal's serum K level by 1.5, 3.0 and 6.0 meq/l. Recordings of CBF, AP, and PP were taken 2 min-

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