constant was higher than could be accounted for on the basis of the incidence of spontaneous tumors in this strain. The hypothesis implies an average of more than 3 tumors at the zero dose, whereas the observed average was .29. This increased response is even more difficult to explain than the decreased response shown for the single-action hypothesis. The two-action hypothesis implies that the curve is concave upward, which would give a rapidly increasing number of tumors at the larger doses. Instead, at the higher doses the curve probably would be found to flatten out. Since there is also a time element involved if the actual response curve is sigmoid, observing the tumors earlier might give an upward curve, whereas later observations might give a downward curve. This requires further investigation.

Data on pulmonary tumors induced in mice with methylcholanthrene by Shimkin and McClelland (*8*) were examined to see if the linear relationship between the number of nodules and the dose was also present in their experiment. Such a linear relationship could not be established, there being significant departure from linearity when the χ^2 test was used. However, there were not as many animals per group in their experiment as in the groups reported herein, and the technique used in counting was not the same for the two experiments. In their experiment the nodules were counted in lungs that previously had been fixed in Tellyesniczky's fluid, and apparently the counting was done without the aid of the dissecting microscope. Their data did not fit a parabolic curve such as reported by Charles and Luce-Clausen either.

It was possible, however, to estimate the "net disposal" constant (k) for Shimkin and McClelland's data such as was estimated from our own experiment. Their data were in three series, with observations made after 8, 13, and 18 weeks. For these series, k was .047 mg, .040 mg, and .047 mg methylcholanthrene, respectively. These constants are more nearly alike than would have been expected from the variance of k which is implied by the mathematics.

Although the data of Shimkin and McClelland do not support, they do not oppose our own data, which with the analysis thereof would fit the postulate that the induction of pulmonary tumors in the mouse is the result of a single change in the cell. If this were a genic change it would be assumed to be a dominant mutation.

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Ascorbic Acid and the Oxidation of Tyrosine

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Many lines of evidence have suggested that ascorbic acid is involved in the biological oxidation of tyrosine. Studies with rat liver homogenate preparations (*1*) and acetone powder extracts (*2*) have indicated that the effect of ascorbic acid in the conversion of tyrosine to acetoacetic acid is primarily upon the first oxidative step of this metabolic pathway, the oxidation of *p*-hydroxyphenylpyruvic acid. Recently it has been proposed that ascorbic acid is a cofactor for the enzyme catalyzing this oxidative step (*3*). In the present communication, evidence will be presented that ascorbic acid may have a less specific role and can be replaced by a number of structurally unrelated compounds that are susceptible to oxido-reduction.

In experiments with extracts of liver acetone powder of rat, rabbit, and dog, it has been reported that tyrosine, *p*-hydroxyphenylpyruvic acid, 2,5-dihydroxyphenylpyruvic acid, and homogentisic acid oxidized enzymatically to acetoacetic acid (*2, 4*). The addition of ascorbic acid to this preparation increases the oxidation of tyrosine or *p*-hydroxyphenylpyruvic acid but has no effect upon the oxidation of 2,5-dihydroxyphenylpyruvic acid and homogentisic acid (*2*). The effect of ascorbic acid on the oxidation of *p*-hydroxyphenylpyruvic acid can be studied by using either *p*-hydroxyphenylpyruvic acid as the substrate or by using tyrosine with α-ketoglutarate to generate *p*-hydroxyphenylpyruvic acid via the tyrosine transaminase system present in the extract. The effect of ascorbic acid was established by measuring the rate of disappearance of substrate and by following the reaction manometrically in the Warburg apparatus. When 10 μM of L-tyrosine was used as the substrate with the liver extract preparation, the addition of ascorbic acid increased the oxidation of tyrosine only when α-ketoglutarate was also present, as shown in Fig. 1. A concentration of 0.001 *M* (4 μM) ascorbic acid was found to be sufficient to produce a maximal stimulatory effect upon the oxidation. Further increase in the concentration of ascorbic acid did not increase the initial rate of oxygen uptake, nor did it alter the products of the reaction. Suboptimal concentrations of ascorbic acid resulted in less tyrosine being converted to acetoacetic acid.

The preparation of the acetone powder removes part of the ascorbic acid orginally present in the tissue. The residual amount remaining in the crude powder extract is sufficient, however, to maintain appreciable oxidative activity. Dialysis of the crude

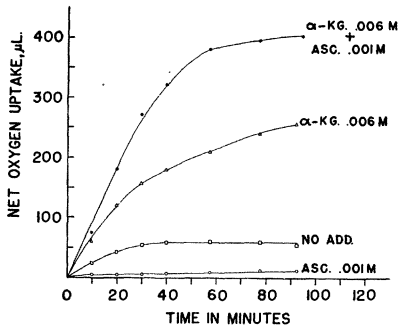

FIG. 1. The stimulation of L-tyrosine oxidation by ascorbic acid (ASC) in the presence of α-ketoglutarate (*α-KG*). Flask contents: 2.0 ml rabbit liver powder extract; 1.0 ml pyrophosphate buffer; 0.3 ml α-ketoglutarate (20 μM) or 0.3 ml of H_2O. The side arms contained 10 μM L-tyrosine in 0.5 ml phosphate buffer (buffer alone was used in the control flasks), and 0.2 ml ascorbic acid (4.0 μM). Total volume, 4.0 ml. Both α-ketoglutarate and ascorbic acid were omitted in the no-addition flasks (*No ADD.*).

powder extract greatly reduces the ability to oxidize *p*-hydroxyphenylpyruvic acid, but this activity is nearly completely restored by the addition of ascorbic acid to the dialyzed extract system.

Considerably less than stoichiometric amounts of ascorbic acid are needed for the oxidation of tyrosine. Titration with 2,6-dichlorophenolindophenol at the end of the incubation period has shown that nearly half the added ascorbic acid remains in the reduced form. Since the titration values are the same for the control flasks and the ones in which tyrosine was oxidized, it appears that no net consumption of ascorbic acid is required for the oxidation of tyrosine.

Although these results are in agreement with the theory that ascorbic acid acts as a cofactor for the enzyme system catalyzing the oxidation of *p*-hydroxyphenylpyruvic acid, the data presented below suggest that ascorbic acid may act in a less specific manner.

Knox (*1*) observed in experiments with the rat liver homogenate preparation, that D-isoascorbic acid also increases the oxidation of tyrosine. We have tested several compounds using the acetone powder extract preparation and have found that D-isoascorbic acid, D-ascorbic acid, and hydroquinone are just as effective as L-ascorbic acid on a molar basis. Homogentisic acid, *p*-aminophenol, *p*-phenylenediamine, and 2,6-dichlorophenolindophenol also increase tyrosine oxidation but are less effective than ascorbic acid. On the other hand, catechol, resorcinol, 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylalanine, dihydroxymaleic acid, cysteine, and glutathione are unable to replace ascorbic acid or to supplement a suboptimal concentration of ascorbic acid. The oxidized forms of ascorbic acid or hydroquinone (dehydroascorbic acid or quinone) are nearly as effective as the reduced forms. This would be expected if these compounds undergo cyclic oxidation and reduction during the oxidation of tyrosine.

The observation that several compounds are able to stimulate the oxidation of *p*-hydroxyphenylpyruvic acid suggests that the requirement is one for a compound having the proper oxidation-reduction potential. Whether these compounds found to be active in place of ascorbic acid function by protecting the small amount of ascorbic acid present in the powder extract or completely replace ascorbic acid in this system cannot be determined without further purification of the enzyme system involved.

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Comments and Communications

Zoological Nomenclature

NOTICE is hereby given that, as from June 29, 1953, the International Commission on Zoological Nomenclature will start to vote on the following cases involving the possible use of its plenary powers for the purposes specified against each entry. Full particulars of these cases were published on Dec. 29, 1952, in the *Bulletin of Zoological Nomenclature* in Double-Part 4/5 of Vol. 9. (1) *Astacus Fabricius,* 1775 (Class Crustacea, Order Decapoda), validation of (correction of an error in Opinion 104); (2) *Favus* Lanchester, 1900 (Cl. Crustacea, Ord. Decapoda), validation of (correction of an error in Opinion 73); (3) *flavipes* Olivier, 1795, *Dytiscus* (Cl. Insecta, Ord. Coleoptera), validation of, by the suppression of *flavipes* Fabricius, 1792, *Dytiscus.*

Comments on the above cases should be sent as soon as possible to the undersigned.

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Scientific Conferences and Papers

IT SEEMS worth while at this time, when so many conferences on such a variety of subjects are scheduled, to review the fundamental purposes of a scientific conference and the methods of best achieving these ends.

The principal objective of a scientific conference