with the active centre of the enzyme, but in the case of urease the suramin appears to act by 'bridging' across the active centre. In both cases the sulphonic acid groups of suramin are concerned with the reaction with the enzyme.

7. Many enzymes are, like urease, inhibited by suramin in acid solution but not in neutral or alkaline solution. In these cases the inhibition-pH curve usually falls sharply at about the isoelectric point of the enzyme.

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Biochemical Studies of Toxic Agents

2. THE METABOLISM OF 2-NAPHTHYLAMINE AND 2-ACETAMIDONAPHTHALENE

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Exposure to certain intermediates used in the manufacture of dyestuffs has long been recognized as a factor in the production of bladder tumours in workers in the industry. Among the various compounds used in the synthesis of dyestuffs, only 2-naphthylamine appears to have been shown experimentally to give rise to tumours located predominantly in the bladder. The first well established experimental demonstration of this carcinogenic

action of 2-naphthylamine was that of Hueper, Wiley & Wolfe (1938), who obtained papillomas and carcinomas of the bladder in thirteen out of sixteen female dogs which had received commercial 2-naphthylamine by subcutaneous injection and oral administration for a period of 20–26 months. Later, Bonser (1943) described the production of bladder tumours in dogs following prolonged oral administration of purified 2-naphthylamine. The fact that the tumours to which this amine gives rise in man and in experimental animals occur almost exclusively in the bladder has led to the suggestion that the carcinogenic agent is excreted in the urine and that it is

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either 2-naphthylamine itself or a metabolite of this compound. Because of this possibility, considerable interest attaches to the nature of the excretory products to which 2-naphthylamine gives rise.

Kuchenbecker (1920), on the basis of qualitative tests, reported that in dogs 2-naphthylamine is excreted in the urine unchanged. Engel (1920, 1924) stated that only traces of the unchanged compound are excreted in the urine of dogs dosed with 2-naphthylamine, and considered that oxidation of the compound to 2-amino-1-naphthol takes place followed by conjugation of this compound with sulphuric acid and glucuronic acid. The metabolic formation of the sulphuric acid derivative was established by Wiley (1938), who isolated 2-amino-1-naphthylsulphuric acid from the urine of dogs to which he had given 2-naphthylamine by mouth.

A study of the metabolism of 2-naphthylamine was made by Dobriner, Hofmann & Rhoads (1941), who administered a solution of the compound to rats, rabbits and monkeys by subcutaneous injection. From the urine of all three species they isolated 2-naphthylamine, 2-acetamidonaphthalene, and 2-acetamido-6-hydroxynaphthalene. By spectroscopic methods they obtained evidence which led them to suggest that the urine contained free 2-amino-6-hydroxynaphthalene and that this compound or its acetyl derivative or both compounds were excreted in part in conjugation with sulphuric acid and glucuronic acid. As a result of these various investigations it appeared, therefore, that in the dog oxidation of 2-naphthylamine takes place in the 1-position, whereas in the rat, rabbit and monkey this amine is oxidized in the 6-position.

In the present work the metabolism of 2-naphthylamine was studied in the rat following administration of the amine by subcutaneous injection or by stomach tube. 2-Amino-1-naphthylsulphuric acid was isolated from the urine of the dosed animals, and it was thus established that formation of this compound from 2-naphthylamine takes place in the rat as well as in the dog. In confirmation of the work of Dobriner *et al.* (1941) it was found that the administration of 2-naphthylamine to rats by subcutaneous injection is followed by the excretion of 2-naphthylamine and 2-acetamido-6-hydroxynaphthalene in the urine. These compounds were also isolated from the urine of rats following the administration of 2-naphthylamine by stomach tube. Traces of a compound which appeared to be 2-acetamidonaphthalene were separated from the ether extract of the urine of the dosed animals, but the amounts obtained were insufficient to permit identification with certainty.

The fact that 2-naphthylamine gives rise to formation of 2-acetamido-6-hydroxynaphthalene in the rat led to a study being made of the metabolism of 2-acetamidonaphthalene. This compound was administered to rats by subcutaneous injection and by

mouth, and 2-acetamido-6-hydroxynaphthalene was isolated from the urine of the dosed animals. The possible significance of this finding in relation to the metabolism of 2-naphthylamine is discussed later.

THE METABOLISM OF 2-NAPHTHYLAMINE

Groups of white rats consisting of equal numbers of male and female animals weighing between 200 and 300 g. were used in the experiments. The rats were kept in metabolism cages which permitted the separate collection of urine and faeces. They were fed in separate cages for two 1 hr. periods each day and received a diet of Master Fox Breeding Ration (Toronto Elevators Ltd.) supplemented with fresh milk and brown bread. Water was available to the animals while they were in the metabolism cages.

The 2-naphthylamine was administered by stomach tube or by subcutaneous injection. A group of twelve rats was used in each experiment in which the compound was given by stomach tube and each rat received daily on 4 successive days 1.0 ml. of a 1% starch solution containing 0.1 g. of 2-naphthylamine. The total amount of 2-naphthylamine administered in each experiment was 4.8 g. When the compound was given by subcutaneous injection each rat in a group of twenty-four animals received on each of 2 successive days 0.5 ml. of a 5% (w/v) solution of 2-naphthylamine in olive oil. The total amount of 2-naphthylamine given by subcutaneous injection in each experiment was 1.2 g. In all experiments the urine was collected from the time the animals were given the first dose of the compound until 2 days after the last dose. The urine was collected daily and stored in the refrigerator. As a measure of protection against the oxidation of the urinary constituents, about 0.1 g. of sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) was placed in the urine collection vessel of each metabolism cage at the beginning of each daily collection period.

Extraction of the urine. The urine (pH 6-7) in each experiment was extracted with 250 ml. of peroxide-free ether in a continuous extractor for a total of 30 hr. The ether extract was shaken with small portions of N-HCl and then with distilled water. The acid and aqueous extracts were combined and dissolved ether was removed under reduced pressure ('acid extract of the ether extract of the urine'). The ether extract was next shaken with small portions of N-NaOH and then with distilled water. The alkaline and aqueous extracts were combined and the dissolved ether was removed under reduced pressure ('alkali extract of the ether extract of the urine'). The ether extract, after being treated in the manner just described, was evaporated to dryness under reduced pressure.

The urine, after ether extraction, was treated as follows. Dissolved ether was removed under reduced pressure and the urine was brought to pH 2 by the addition of conc. HCl . It was then shaken with one 200 ml. portion and five 100 ml. portions of *n*-butanol saturated with water. The emulsions which formed were broken by centrifuging. The butanol extracts were combined, and a slight excess of NH_3 was added ('butanol extract of the acidified urine'). In an experiment in this laboratory in which ^{35}S (as Na_2SO_4) was injected intraperitoneally into rats at the same time as they were dosed with 2-naphthylamine by subcutaneous injection it was found that butanol extraction of the urine under the above conditions removed almost all the ^{35}S present as ethereal sulphate (Laidlaw, 1947).

Isolation of 2-naphthylamine

The acid extract of the ether extract of the urine was made alkaline by the addition of NaOH solution and left in the refrigerator overnight. The deep-purple precipitate which formed was filtered, washed with water, and dried over P_2O_5 *in vacuo*. The dried product was sublimed under reduced pressure and yielded a crystalline sublimate, m.p. 110–111°, mixed m.p. with 2-naphthylamine 110–111° (these and other m.p.'s reported herein are uncorrected unless otherwise stated). (Found: C, 83.6; H, 6.3. Calc. for $C_{10}H_9N$: C, 83.9; H, 6.3%.)

Some of the compound isolated from urine (0.050 g.) was dissolved in 2 ml. of dry pyridine and 0.1 ml. of acetic anhydride was added. The mixture was refluxed for 15 min., poured into 10 ml. of ice water, stirred well, and left in the refrigerator overnight. The precipitate which formed was filtered and washed first with dilute HCl and then with water. The product was dried over P_2O_5 and was then sublimed under reduced pressure. A crystalline sublimate weighing 0.032 g. was obtained, m.p. 131–132°, mixed m.p. with 2-acetamidonaphthalene 131–132°. (Found: C, 77.7; H, 5.9. Calc. for $C_{12}H_{11}ON$: C, 77.8; H, 6.0%.)

When the rats were dosed with 2-naphthylamine by stomach tube the amounts of the amine isolated from the urine were 0.134 and 0.220 g., i.e. 2.8 and 4.6%, respectively, of the 2-naphthylamine administered. In experiments in which the amine was injected subcutaneously into rats the amounts isolated weighed 0.078, 0.068 and 0.051 g., and these corresponded to 6.5, 5.7 and 4.3%, respectively, of the 2-naphthylamine injected.

Isolation of 2-acetamido-6-hydroxynaphthalene

The alkali extract of the ether extract of the urine was acidified with conc. HCl and left in the refrigerator overnight. The precipitate which formed was filtered and washed with distilled water. The solid material, which was a dark brown colour, was dissolved in 95% ethanol. The solution was filtered and the filtrate was heated with charcoal. After removal of the charcoal, the solution was evaporated to a small volume and hot water was added to the warm ethanol solution until it became cloudy. The solution, after being allowed to cool, was placed in the refrigerator overnight and colourless needle-shaped crystals separated. These were collected by filtration and dried, m.p. 217–218°. (Found: C, 71.5; H, 5.1. Calc. for $C_{12}H_{11}O_2N$: C, 71.6; H, 5.5%.)

The compound was insoluble in acid and soluble in alkali. When diazobenzenesulphonic acid was added to an alkaline solution of the compound a deep red colour was produced. The compound gave a positive diazo test for the presence of an amino group only after it had been hydrolysed by heating with acid. The properties and analysis of the compound suggested that it was 2-acetamido-6-hydroxynaphthalene, a derivative which had been isolated by Dobriner *et al.* (1941) from the urine of rats, rabbits and monkeys which had received 2-naphthylamine by subcutaneous injection.

Some of the compound isolated from urine was converted to its methoxy derivative in the following manner. The compound (0.050 g.) was dissolved in 5.0 ml. of 20% (w/v) NaOH solution and while the solution was being warmed and stirred, 1.0 ml. of dimethyl sulphate was added in small portions. When all the dimethyl sulphate had been added the solution was warmed on a steam bath for 30 min. It was

then placed in the refrigerator overnight and the precipitate which formed was filtered and washed with water. The product was crystallized from aqueous ethanol, filtered and dried; yield 0.033 g. (Found: C, 72.6; H, 6.1. Calc. for $C_{13}H_{13}O_2N$: C, 72.5; H, 6.1%.) The methoxy derivative melted at 160–161°; Windaus (1924) reported 159–160° for the melting point of 2-acetamido-6-methoxynaphthalene.

From the findings which have been described it was concluded that the compound isolated from the alkaline extract of the ether extract of the urine was 2-acetamido-6-hydroxynaphthalene. The amount of the compound isolated in an experiment in which 2-naphthylamine was given by stomach tube was 0.071 g., and this corresponded to 1.1% of the 2-naphthylamine administered. The combined alkaline extracts from four experiments in which 2-naphthylamine was given by subcutaneous injection yielded 0.136 g. of 2-acetamido-6-hydroxynaphthalene. This corresponded to 2.0% of the 2-naphthylamine injected.

Isolation of 2-amino-1-naphthylsulphuric acid

The butanol extract of the acidified urine was evaporated to dryness under reduced pressure at a temperature which did not exceed 45° (as described earlier, a slight excess of NH_3 had been added to this extract). The dry residue was taken up in small portions of a mixture of 90 vol. ethanol and 10 vol. conc. HCl and the acid-ethanol solution (total vol. 65 ml.) was left in the refrigerator for several hours. The light-purple coloured precipitate which formed was filtered and washed with 2 ml. portions of the acid-ethanol solution until colourless washings were obtained. The product was dried *in vacuo* over P_2O_5 and dissolved in *N*-NaOH. The solution was filtered and the filtrate was acidified with conc. HCl. The precipitate which formed was filtered, washed well with water, and was then dissolved in boiling water. The solution was heated with charcoal and filtered, and the filtrate was left in the refrigerator overnight. A pale pink coloured precipitate formed which was filtered and dried over P_2O_5 *in vacuo*. The product darkened at 215° and melted with decomposition at 222–223°. The m.p. (corr.) of the 2-amino-1-naphthylsulphuric acid isolated by Wiley (1938) from the urine of dogs dosed with 2-naphthylamine was 224°. (Found: C, 50.4; H, 3.8; S, 13.7. Calc. for $C_{10}H_8O_4NS$: C, 50.2; H, 3.8; S, 13.4%.) A hot aqueous solution of the compound gave no precipitate with $BaCl_2$ solution, but after hydrolysis of the compound with HCl a white precipitate formed on the addition of $BaCl_2$ solution.

Some of the compound (0.300 g.) was refluxed with 20 ml. of 5*N*-HCl for 6 hr. A small amount of charcoal was added to the hot solution and the mixture was filtered while hot. The filtrate was evaporated under reduced pressure to 5 ml. and chilled in a freezing mixture. The crystalline precipitate which formed was filtered and dried over P_2O_5 *in vacuo*; yield 0.196 g.; m.p. 252–253° (decomp.). (Found: C, 61.6; H, 5.0. Calc. for $C_{10}H_{10}ONCl$: C, 61.4; H, 5.2%.) A portion of the hydrolysis product (0.052 g.) was dissolved in 2 ml. of pyridine, 0.1 ml. of acetic anhydride was added, and the mixture was refluxed for 20 min. The hot solution was poured into 10 ml. of ice water, the mixture was stirred until crystallization started and was then left in the refrigerator overnight. The crystalline product was filtered, washed with dilute HCl and with water, and recrystallized from aqueous ethanol. The product, which weighed 0.017 g., was soluble in alkali and insoluble in acid, and an alkaline solution gave a red colour with diazobenzene-

sulphonic acid. The melting point of the compound was 127–128°; the melting point of 2-acetamido-1-hydroxynaphthalene is 128–129° (Grandmougin, 1906).

On the basis of these findings it is concluded that the compound isolated from urine was 2-amino-1-naphthylsulphuric acid. The amount of the compound obtained from the urine of rats dosed with 2-naphthylamine by stomach tube was 0.429 g., and this was equivalent to 5.3% of the compound administered. In two experiments in which the amine was injected subcutaneously the amounts of the H_2SO_4 derivative isolated from the urine were 0.145 and 0.102 g., and these corresponded to 7.2 and 5.1%, respectively, of the compound administered.

THE METABOLISM OF 2-ACETAMIDONAPHTHALENE

2-Acetamidonaphthalene was administered to rats by stomach tube and by subcutaneous injection. The general experimental conditions were similar to those which have already been described for the experiments in which 2-naphthylamine was administered to rats. When 2-acetamidonaphthalene was given by stomach tube each rat in a group of twelve received, on each of 4 consecutive days, 1 ml. of a 1% starch solution containing 0.1 g. of the compound. When the compound was injected subcutaneously, each animal in a group of twelve received daily on 4 consecutive days 1 ml. of a 10% solution (w/v) of the compound in propylene glycol at 38–40°. It was necessary to maintain the solution at this temperature in order to avoid precipitation of the compound.

The urine was extracted with ether and the ether extract was fractionated by the procedures described for the experiments on the metabolism of 2-naphthylamine.

Isolation of 2-acetamidonaphthalene

After the ether extract of the urine of the rats dosed with 2-acetamidonaphthalene by stomach tube had been shaken with *N*-HCl and *N*-NaOH, and had been washed with water, it was evaporated to dryness under reduced pressure. The residue was extracted with boiling light petroleum (60–80°) and the extract was evaporated to dryness. The residue thus obtained was dissolved in ethanol. The solution was heated with charcoal, filtered and evaporated to dryness. The residue was dissolved in hot light petroleum (60–80°) and when the solution was evaporated to small volume it yielded 0.030 g. of crystalline material, m.p. 130–131°, mixed m.p. with 2-acetamidonaphthalene 131–132°. The amount of the compound isolated corresponded to 0.6% of that administered. Insufficient material for identification purposes was obtained when the procedure just described was applied to the urine of rats dosed by subcutaneous injection with 2-acetamidonaphthalene.

Isolation of 2-naphthylamine

From the urine of rats dosed with 2-acetamidonaphthalene by stomach tube a very small amount of 2-naphthylamine was obtained by the procedure used to isolate this compound from the urine of rats dosed with 2-naphthylamine. In two experiments, in each of which the rats received 4.8 g. of 2-acetamidonaphthalene, the total amount of 2-naphthylamine isolated from the urine was 0.003 g. The m.p. and mixed m.p. of the isolated compound were

109–111°. It was not found possible to obtain the amine from the urine of rats dosed with 2-acetamidonaphthalene by subcutaneous injection.

Isolation of 2-acetamido-6-hydroxynaphthalene

The alkali extract of the ether extract of the urine of rats dosed with 2-acetamidonaphthalene was fractionated by the method already described for the isolation of 2-acetamido-6-hydroxynaphthalene from the urine of rats dosed with 2-naphthylamine. This yielded a crystalline product, m.p. 217–218°. (Found: C, 71.5; H, 5.7. Calc. for $C_{12}H_{11}O_2N$: C, 71.6; H, 5.5%.)

The methoxy derivative was prepared by the method already described and 0.035 g., m.p. 161–162°, was obtained from 0.050 g. of the compound isolated from urine. (Found: C, 72.4; H, 6.2. Calc. for $C_{13}H_{13}O_2N$: C, 72.5; H, 6.1%.)

It is concluded that the compound obtained from the urine was 2-acetamido-6-hydroxynaphthalene. When the compound was mixed with 2-acetamido-6-hydroxynaphthalene isolated from the urine of rats dosed with 2-naphthylamine the melting point was not depressed. Furthermore, the melting point of a mixture of the methoxy derivatives of these two products was not depressed.

The amount of 2-acetamido-6-hydroxynaphthalene obtained from the urine of rats dosed with 2-acetamidonaphthalene by stomach tube was 0.307 g. and this was equivalent to 4.5% of the compound administered. When the acetamido compound was injected subcutaneously, 0.381 g. of the hydroxy derivative was isolated, and this corresponded to 5.6% of the compound injected.

DISCUSSION

The urine excreted by rats dosed with 2-naphthylamine by stomach tube or by subcutaneous injection was found to contain 2-naphthylamine, 2-acetamido-6-hydroxynaphthalene and 2-amino-1-naphthylsulphuric acid. Dobriner *et al.* (1941) were able to isolate 2-acetamidonaphthalene from the urine of rats dosed with 2-naphthylamine by subcutaneous injection. If this compound was a constituent of the urine in our experiments the amounts present were probably very small. The excretion of 2-amino-1-naphthylsulphuric acid by rats following the administration of 2-naphthylamine has not been reported previously, although this compound was shown by Wiley (1938) to be present in the urine of dogs dosed with 2-naphthylamine. The compounds isolated from the urine accounted for the following percentages of the 2-naphthylamine administered: (1) by subcutaneous injection: 4.3–6.5% as 2-naphthylamine, 2.0% as 2-acetamido-6-hydroxynaphthalene, 5.1–7.2% as 2-amino-1-naphthylsulphuric acid; (2) by stomach tube: 2.8–4.6% as 2-naphthylamine, 1.1% as 2-acetamido-6-hydroxynaphthalene, and 5.3% as 2-amino-1-naphthylsulphuric acid. Wiley (1938) found an increase in the organic sulphate content of the urine of dogs corresponding to 52% of the 2-naphthylamine fed, but he did not report the amount of 2-amino-1-naphthylsulphuric acid which he isolated from urine, and Dobriner *et al.* (1941) did not

report the amounts of the metabolites of 2-naphthylamine which they isolated in their experiments.

The fact that 2-acetamido-6-hydroxynaphthalene and 2-amino-1-naphthylsulphuric acid are excreted

lene, for it cannot be assumed that the fate of 2-acetamidonaphthalene formed in the organism is the same as that of administered 2-acetamidonaphthalene.

Conversions which have been shown to take place in the rat are indicated by solid arrows; possible intermediary reactions are indicated by broken arrows.

in the urine of rats dosed with 2-naphthylamine raises the question of whether 2-amino-6-hydroxynaphthalene and 2-amino-1-hydroxynaphthalene are formed during the metabolism of 2-naphthylamine.

The metabolic formation of 2-acetamido-6-hydroxynaphthalene from 2-naphthylamine might take place: (1) by oxidation of 2-naphthylamine to 2-amino-6-hydroxynaphthalene followed by acetylation of the latter compound; or (2) by acetylation of 2-naphthylamine followed by the oxidation of 2-acetamidonaphthalene to 2-acetamido-6-hydroxynaphthalene. Neither free 2-amino-6-hydroxynaphthalene nor its conjugate with sulphuric acid or glucuronic acid has yet been isolated from the urine of animals dosed with 2-naphthylamine, although Dobriner *et al.* (1941) obtained spectroscopic evidence which suggested that one or more of these compounds may have been present in the urine obtained in their experiments. On the other hand, it has been shown in the present work that the administration of 2-acetamidonaphthalene to rats by subcutaneous injection or by stomach tube is followed by the excretion of 2-acetamido-6-hydroxynaphthalene. Furthermore, although no appreciable amount of 2-acetamidonaphthalene appeared to be present in the urine in our experiments with rats dosed with 2-naphthylamine, this compound has been shown by Dobriner *et al.* (1941) to be excreted by rats dosed with the amine. There is evidence, therefore, that in the rat 2-naphthylamine undergoes acetylation, and that 2-acetamidonaphthalene is converted to 2-acetamido-6-hydroxynaphthalene. It cannot be said, however, that these observations establish that formation of 2-acetamidonaphthalene is a necessary step in the metabolic conversion of 2-naphthylamine to 2-acetamido-6-hydroxynaphtha-

The isolation of 2-amino-1-naphthylsulphuric acid from the urine of the dosed animals suggests that 2-naphthylamine is oxidized in the rat to 2-amino-1-hydroxynaphthalene which then undergoes conjugation with sulphuric acid. This is in accordance with commonly accepted views concerning the mode of formation of 'ethereal' sulphates. If formation of 2-amino-1-hydroxynaphthalene takes place it is probable that some of this compound undergoes conjugation with glucuronic acid with the formation of 2-amino-1-naphthylglucuronide, a compound which has not yet been shown to be present in the urine of animals dosed with 2-naphthylamine. As a result of their work on the metabolism of aniline and *p*-aminophenol in rats, Elson, Goulden & Warren (1946) have suggested that 'ethereal sulphate formation can take place directly in the aromatic nucleus without preliminary formation of free phenolic compounds'. If the mechanism described by Elson *et al.* operates in the production of 2-amino-1-naphthylsulphuric acid from 2-naphthylamine in the rat, the intermediate formation of free 2-amino-1-hydroxynaphthalene need not take place.

In view of the possibility that bladder tumours may be produced in the dog by a metabolite of 2-naphthylamine, it is interesting to note that prior to the present work it was known that 2-naphthylamine is converted to 2-amino-1-naphthylsulphuric acid in the dog, whereas this change had not been shown to occur in the rat. It has now been established that conversion of 2-naphthylamine to 2-amino-1-naphthylsulphuric acid occurs in the rat as well as in the dog. In this respect, therefore, the metabolism of 2-naphthylamine in the rat resembles its metabolism in the dog, although administration of 2-naphthylamine does not give rise to bladder tumours in the rat (Greenstein, 1947).

SUMMARY

1. The administration of 2-naphthylamine to rats, either by subcutaneous injection or by stomach tube, is followed by the excretion in the urine of 2-naphthylamine, 2-acetamido-6-hydroxynaphthalene and 2-amino-1-naphthylsulphuric acid.

2. When rats are dosed with 2-acetamidonaphthalene by subcutaneous injection or by stomach tube, the urine contains 2-acetamido-6-hydroxynaphthalene. The urine of rats given 2-acetamidonaphthalene by stomach tube contains small amounts of the unchanged compound and 2-naphthylamine, but these compounds have not been found in the urine of rats dosed with 2-acetamidonaphthalene by subcutaneous injection.

3. The possible steps in the metabolic conversion of 2-naphthylamine to the derivatives excreted in the urine are discussed.

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The Fermentation Process in Tea Manufacture

11. OXIDATION OF SUBSTRATES BY TEA OXIDASE

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Considerable differences in opinion are on record as to what substrates are acted upon by the tea oxidase, and also as to their relative rates of oxidation. Thus Deb & Roberts (1940) claimed that, in addition to *o*-dihydroxypolyphenols, *p*-phenylenediamine, ascorbic acid and quinol are also oxidized, whereas Lamb & Sreerangachar (1940*a*) stated that *o*-dihydroxyphenols only are oxidized. Li & Bonner (1947) claim to have shown that although *p*-phenylenediamine is oxidized, the oxidation is catalysed by a water-soluble thermostable system. As originally suggested by Lamb & Sreerangachar (1940*a, b*), it is now clear that some of these apparent differences in specificity are due to adsorption of substrates by crude enzyme preparations. Elimination of such impurities results in the loss of the enzyme's ability to catalyse oxidation of quinol and ascorbic acid, but

not of *p*-phenylenediamine. The use of enzymes containing adsorbed polyphenols is also shown to affect results obtained with catechol as a substrate.

Again, whereas Harrison & Roberts (1939), using substrates at 0.01*M*-concentration, showed that tea catechins (tannins) are oxidized about three times as rapidly as catechol, Sreerangachar's (1943*b*) manometric work indicated that catechol is oxidized faster than tea catechins, when both substrates are at 0.05*M*-concentration. On the other hand, his results (1943*a*) with the ascorbic acid oxidation method, indicate that catechins are oxidized faster than catechol at all concentrations investigated.

In order to reconcile the above differences, a detailed investigation of substrate specificity has been carried out, using several different types of enzyme preparation.