XII. OXIDISING ENZYMES. IV. THE DISTRIBUTION OF OXIDISING ENZYMES AMONG THE HIGHER PLANTS.

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This and the following paper are a continuation of investigations upon the oxidising enzymes of fruits undertaken for the Food Investigation Board of the Department of Scientific and Industrial Research.

In a previous paper [Onslow, 1920] evidence has been given in favour of the view that many plants contain a representative of a class of enzymes which have been termed oxygenases. It is suggested that these enzymes catalyse the autoxidation of aromatic substances having the dihydroxy grouping of catechol, that is two hydroxyl groups in the ortho-position. Such substances are known to be widely distributed in plants, and certain compounds of this character, such as catechol, caffeic acid, protocatechuic acid, hydrocaffeic acid, etc. have been identified in various genera and species.

The existence of an oxygenase and its aromatic substrate has already been demonstrated in several fruits [Onslow, 1920]. With a view to finding out how general the distribution of these enzymes might be, an examination has been made of plants selected from a number of orders of the Angiosperms. In the case of many orders only one representative has been examined, as the object in view was to ascertain how widely oxygenases are distributed; in certain cases, however, a number of genera in one order have been tested.

Since the reactions of oxidising enzymes may be greatly interfered with by sugars, tannins, organic acids and other substances, the following procedure, already described [Onslow, 1920], has been adopted in making the test. The plant tissue is well pounded in a mortar with 96–98 % alcohol and the mixture filtered on a filter pump. The process is then repeated once or twice with the residue on the filter. In this way the sugars, tannins, etc., are removed, but the enzymes are precipitated and retained in the residue. Previous observations have shown, however, that in some cases the enzyme is not readily extracted from such a residue by water. Hence in testing, the residue itself is divided into two portions in two small evaporating basins. One portion is stirred with dilute catechol solution, the other with water. In cases where oxygenase is present, browning occurs in the catechol solution and is followed by a blue colour on addition of guaiacum solution. (Before use, the alcoholic solution of guaiacum should be well boiled with a little Merck's charcoal and filtered. This removes any peroxides which may have formed by autoxidation of the guaiacum.)

If no reaction occurs, the control dish is tested with guaiacum and hydrogen peroxide which will determine whether a peroxidase is present or not. If the peroxidase should be absent, which is a rare occurrence, it should be borne in mind that the reaction for oxygenase cannot take place, even though the oxygenase enzyme may be present.

The total number of species examined was 320 and these constitute a selection from the whole of the group of Angiosperms. They represent, moreover, 180 Natural Orders and 309 genera. Of the above species, 16 (*i.e.* 5 %) representing 16 genera and 13 Natural Orders, failed to give a peroxidase reaction with hydrogen peroxide and guaiacum. In order to ascertain satisfactorily whether they contain oxygenase by the catechol-guaiacum test, it would be necessary in these cases to add peroxidase from another source. This has not been tried, so that in estimating the percentage of "positive orders" (*i.e.* those containing oxygenase) these 16 species must be eliminated. Some of the latter occur in Natural Orders (marked * in the following list) of which other representatives have been examined, so that in fact only 7 orders remain to be cancelled on the above grounds. They are Bruniaceae, Ebenaceae, Empetraceae, Frankeniaceae, Myrsinaceae, Pyrolaceae and Sarraceniaceae. It should strictly be understood that further observations on these species, or on additional genera and species, may show that these orders might be ranked as positive and they are merely eliminated from the present set of observations.

Taking these revised figures of 304 species, 173 Natural Orders and 293 genera, the percentages of positive orders are distributed in the various sub-groups as follows:

Angiosperms:	Percentage of orders examined containing oxygenase
Of the total number of orders (286), 60 % were examined	62
Monocotyledons:	
64 % examined	76
Dicotyledons:	
60 % examined	60
Archichlamydeae:	
57 % examined	51
Sympetalae:	
71 % examined	84

The following is a list of orders examined. Those in italics contain species with oxygenase, *i.e.* are positive orders. The numbers in brackets denote the species examined; the second numbers the species with oxygenase.

Monocotyledoneae.

Pandanales. Typhaceae (1), 0; *Pandanaceae* (2), 2; *Sparganiaceae* (1), 1.

Helobiae. *Potamogetonaceae* (1), 1; Aponogetonaceae (1), 0; Scheuchzeriaceae (1), 0; *Alismaceae* (1), 1; *Butomaceae* (1), 1; *Hydrocharitaceae* (1), 1.

Glumiflorae. *Gramineae* (1), 1; *Cyperaceae* (1), 1.

Principes. *Palmae* (1), 1.

Synanthae. *Cyclanthaceae* (1), 1.

Spathiflorae. *Araceae* (1), 1; *Lemnaceae* (1), 1.

Farinosae. *Bromeliaceae* (2), 1; *Commelinaceae* (1), 1; Pontederiaceae (1), 0.

Liliflorae. *Juncaceae* (1), 1; *Stemonaceae* (1), 1; Liliaceae (3), 0; Amaryllidaceae (2), 0; *Taccaceae* (1), 1; *Dioscoreaceae* (1), 1; Iridaceae (1), 0.

Scitamineae. *Musaceae* (1), 1; *Cannaceae* (1), 1; *Marantaceae* (1), 1.

Microspermae. *Orchidaceae* (1), 1.

Dicotyledoneae (Archichlamydeae).

Verticillatae. *Casuarinaceae* (1), 1.

Piperales. *Piperaceae* (1), 1; *Chloranthaceae* (1), 1.

Salicales. *Salicaceae* (2), 2.

Garryales. *Garryaceae* (1), 1.

Juglandales. *Juglandaceae* (1), 1.

Fagales. Betulaceae (1), 0; Fagaceae (2), 0.

Urticales. *Ulmaceae* (1), 1; *Moraceae* (2), 2; *Urticaceae* (1), 1.

Proteales. *Proteaceae* (1), 1.

Santalales. *Santalaceae* (1), 1; Olacaceae (1), 0; *Loranthaceae* (1), 1.

Aristolochiales. *Aristolochiaceae* (1), 1.

Polygonales. Polygonaceae (1), 0.

Centrospermae. *Chenopodiaceae* (3), 2; Amarantaceae (1), 0; *Nyctaginaceae* (1), 1; Phytolaccaceae (1), 0; *Aizoaceae* (1), 1; *Basellaceae* (1), 1; *Caryophyllaceae* (4), 1.

Ranales. Nymphaeaceae (1), 0; *Ranunculaceae* (11), 9; *Lardizabalaceae* (1), 1; *Berberidaceae** (1), 1; *Menispermaceae* (1), 1; *Magnoliaceae* (1), 1; *Calycanthaceae* (1), 1; *Anonaceae* (1), 1; *Lauraceae* (1), 1.

Rhoeadales. *Papaveraceae* (4), 4; Capparidaceae (1), 0; Cruciferae (8), 0; Resedaceae (1), 0; *Moringaceae* (1), 1.

Sarraceniales. Nepenthaceae (1), 0.

Rosales. Crassulaceae (1), 0; *Saxifragaceae** (3), 1; *Pittosporaceae* (1), 1; Hamamelidaceae (1), 0; Eucommiaceae (1), 0; *Platanaceae* (1), 1; *Rosaceae* (17), 17; Leguminosae (5), 0.

Geraniales. Geraniaceae* (1), 0; Oxalidaceae (1), 0; Tropaeolaceae (1), 0; *Linaceae* (1), 1; *Erythroxylaceae* (1), 1; Zygophyllaceae (1), 0; Rutaceae (2), 0; Simarubaceae (1), 0; Meliaceae (1), 0; Malpighiaceae (1), 0; Polygalaceae (1), 0; Euphorbiaceae (2), 0; *Callitrichaceae* (1), 1.

Sapindales. Buxaceae (1), 0; Coriariaceae (1), 0; *Anacardiaceae* (1), 1; Corynocarpaceae (1), 0; *Aquifoliaceae* (1), 1; Celastraceae (1), 0; Staphyleaceae (1), 0; Aceraceae (1), 0; *Hippocastanaceae* (1), 1; Sapindaceae (1), 0; Melianthaceae (1), 0; Balsaminaceae (1), 0.

Rhamnales. Rhamnaceae (1), 0; *Vitaceae* (2), 2.

Malvales. Elaeocarpaceae (1), 0; Tiliaceae (1), 0; Malvaceae (2), 0; *Sterculiaceae* (2), 2.

Parietales. Dilleniaceae (1), 0; *Eucryphiaceae* (1), 1; *Ochnaceae* (1), 1; *Marcgraviaceae* (1), 1; *Theaceae* (1), 1; *Guttiferae* (1), 1; Tamaricaceae (1), 0; Cistaceae (1), 0; Violaceae (1), 0; *Flacourtiaceae* (1), 1; *Stachyuraceae* (1), 1; Passifloraceae (1), 0; Caricaceae (1), 0; *Loasaceae* (1), 1; Datiscaceae (1), 0; Begoniaceae (1), 0.

Opuntiales. *Cactaceae* (3), 3.

Myrtiflorae. Thymelaeaceae (1), 0; *Elaeagnaceae* (1), 1; Lythraceae (1), 0; Punicaceae (1), 0; *Alangiaceae* (1), 1; Combretaceae (1), 0; Myrtaceae (1), 0; Onagraceae (2), 0; *Hippuridaceae* (1), 1.

Umbelliflorae. *Araliaceae* (2), 2; *Umbelliferae* (5), 5; *Cornaceae* (2), 1.

DICOTYLEDONEAE (Sympetalae).

ERICALES. *Clethraceae* (1), 1; *Ericaceae** (4), 3; Diapensiaceae (1), 0.

PRIMULALES. Theophrastaceae (2), 0; Primulaceae* (3), 0.

PLUMBAGINALES. Plumbaginaceae (1), 0.

EBENALES. Sapotaceae* (1), 0; *Styracaceae* (1), 1.

CONTORTAE. *Oleaceae* (3), 3; *Loganiaceae* (1), 1; *Gentianaceae* (2), 2; *Apocynaceae* (3), 2; *Asclepiadaceae* (1), 1.

TUBIFLORAE. *Convolvulaceae* (1), 1; *Polemoniaceae* (3), 2; *Hydrophyllaceae* (1), 1; *Boraginaceae* (4), 4; *Verbenaceae* (2), 2; *Labiatae* (3), 3; *Nolanaceae* (1), 1; *Solanaceae* (7), 7; *Scrophulariaceae* (14), 14; *Bignoniaceae* (1), 1; *Martyniaceae* (1), 1; *Orobanchaceae* (1), 1; *Gesneriaceae* (6), 6; *Globulariaceae* (1), 1; *Acanthaceae* (1), 1; *Myoporaceae* (1), 1.

PLANTAGINALES. *Plantaginaceae* (1), 1.

RUBIALES. *Rubiaceae* (2), 2; *Caprifoliaceae* (5), 5; *Valerianaceae* (1), 1; *Dipsacaceae* (2), 2.

CUCURBITALES. *Cucurbitaceae* (2), 1.

CAMPANULATAE. Campanulaceae (2), 0; *Compositae* (13), 13.

The observations recorded give an idea of the extent to which the enzymes of the oxygenase type are distributed, and, on the whole, there appears to be a connection between systematic relationship and the presence of oxygenase. Either the presence, or absence, of these enzymes is frequently characteristic of an order. A certain percentage of orders, on the other hand, has been found to contain both positive and negative genera, and this percentage would probably increase if more genera were examined. No definite case of positive and negative species within a genus has been noted, but the data on this point are very limited.

The conclusions which may be drawn are as follows. Enzymes of the oxygenase type are present in the aerial parts of about 62 % of the orders of Flowering Plants examined[1]. Considering the groups in detail, they are widely distributed in the Monocotyledons (76 % of the orders examined); in the Dicotyledons, they are less frequent in the Archichlamydeae (51 %) than in the Sympetalae (84 %). In most of the great cohorts of the Archichlamydeae there is a mixture of positive and negative orders in practically equal numbers, but in the Sympetalae nearly all the orders are positive. It has been suggested, on botanical evidence, that the main lines of evolution among the Sympetalae had their origin among the Archichlamydeae. It would appear that the presence of an oxygenase system is characteristic of plants of the dominating lines of evolution among the Dicotyledons, as practically all the most highly developed orders are positive. Hence these lines may have taken origin from positive stock among the Archichlamydeae.

Though on the whole there is a distinct correlation between presence of oxygenases and systematic relationship, it appears possible that in any genus or species there may be lack of an oxygenase due to some form of metabolism peculiar to that individual. This may not indicate a different origin phylogenetically, as for instance the case of the Campanulaceae, which, though

[1] Many of the remaining orders uninvestigated are rare and it is difficult to obtain representative genera; or they may contain only a few genera. All the larger and widely represented orders have been considered.

negative, are doubtless related to phyla and groups which are characteristically positive.

The extent of the distribution of the catechol substances which act as substrates for the oxygenases has not been so widely investigated. About 30 species, mostly of different genera, which give the oxygenase reaction have been found to contain substances giving the catechol reaction. About 15 species, also chiefly representatives of different genera, which give no oxygenase reaction were found to be devoid of catechol compounds. No case has been noted yet in which oxygenase is present and no catechol compound, though the converse, absence of oxygenase and presence of catechol compound, appears to be true of one or two plants examined.

In the 38 % of negative orders, presumably the oxygenase-catechol system is absent. With respect to this the following points may be considered. It has already been insisted upon repeatedly that water extracts of tissues are usually quite unreliable as material for testing. The same may be true of the actual tissue itself, owing to inhibiting substances present. Even after treatment with alcohol, the readiness with which the tissue residue gives the catechol-guaiacum reaction varies within very wide limits. Hence it is conceivable that in negative species there may still be some inhibitor in the tissue residue, or some inhibiting condition in the experiment, and that the presence of the system cannot be detected by the enzyme method. The enzyme, catalase, might represent such an inhibitor; though almost universally distributed, it might under certain conditions decompose all available peroxide. Experiments to detect such inhibitors have not been successful. Additional determinations of the presence or absence of catechol compounds in negative cases would decide whether the system is absent altogether or not.

Browning and discoloration of the tissues, occurring after death brought about by mechanical injury, chloroform vapour, etc., which was originally thought to accompany the presence of the oxygenase-catechol system, has been found not to be an infallible guide, as it may be inhibited in the same way as the guaiacum reaction. It may also occur in plants in which no oxygenase could be detected (*Vicia*, *Aucuba*) and this point will be referred to again.

It should be borne in mind that the discoloration of tissues, when it does occur, is probably not only due to the oxidation of the catechol compounds but may be augmented by the oxidation of any other aromatic substances present in the tissue. For the system, peroxide-peroxidase, when once established, can oxidise compounds of the most varied types.

Examinations, on a considerable scale, of the guaiacum reaction with plant juices have been conducted by Passerini [1899] and also by Clark [1911]. The former observer examined representatives of about 49, the latter of about 72 orders. Their results agree on the whole with those described here, though fewer direct reactions were obtained on account of fresh juices and water extracts having been employed by them.

In the examination described in the present paper, leaves and leafy shoots were nearly always used as material. Usually the enzyme and substrate are more or less distributed throughout the plant. In some cases as in the raspberry (*Rubus Idaeus*), the blackberry (*R. fruticosus*) and the tomato (*Solanum Lycopersicum*) it was noted that the fruit is negative though the plant is positive. In one case, the fig (*Ficus Carica*), it was noted that the fruit was positive and the leaves negative.

No roots have been examined. Passerini examined various roots and with respect to these organs positive results are recorded by him for species described as negative in the present paper. Possibly root examination might throw further light on distribution.

As far as evidence goes, the conclusion may be drawn that catechol oxygenases, and most probably catechol compounds also, are widely distributed among the Flowering Plants. Their distribution is not apparently correlated with any particular habit or morphological characteristic, as it occurs alike in water and land plants, in trees, shrubs and herbs, in annuals and perennials, in parasites, etc. The only outstanding fact is the almost universal presence of oxygenase in the most highly developed orders, of which the herbaceous, in contrast to the woody, habit is also a characteristic. Hence there is the possibility that there may be a connection between the herbaceous habit and the distribution of the catechol-oxygenases.

The physiological significance of the catechol-oxygenase system is obscure. It is no doubt of the nature of those described by Palladin and others as being involved in respiration processes; such systems, by means of converting molecular into active oxygen, may oxidise substances produced in respiration. If so, one would expect to find the oxygenases universally distributed. No evidence has yet been obtained of an extensive distribution of any other similar system. The bean (*Vicia Faba*) and the Aucuba Laurel (*Aucuba japonica*), however, blacken after death and apparently do not contain a catechol oxygenase. Hence they and other plants may contain a different type of oxidising enzyme.

SUMMARY.

(1) Representatives of a number of orders of the Angiosperms (about 60 % of the total) were examined for an oxygenase which would catalyse the oxidation of catechol. About 62 % of the orders examined were found to contain species with such an enzyme.

(2) With regard to sub-groups of the Angiosperms, such oxygenases are widely distributed in the Monocotyledons, being present in 76 % of the orders examined. In the Dicotyledons such enzymes are less frequent in the Archichlamydeae (51 % of the orders examined) than in the Sympetalae (84 % of the orders examined).

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