INVESTIGATIONS INTO INTERVARIETAL DIFFERENCES OF A CHEMICAL NATURE IN THE POTATO.

by

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Thesis for the Degree of Ph.D.

CONTENTS.

Investigations into Intervarietal Differences of a Chemical Nature in the Potato.

Object of the Investigation.

During the summer months a potato tuber can be accurately identified from a study of the growing plant, each distinct variety giving rise to a characteristic haulm. In the winter, however, the identification of a tuber presents a more difficult problem, and is arrived at by an observation of its visible characters. Such properties as shape of tuber; depth of eye; colour of skin; colour of flesh etc. are not in many cases characteristic of any one variety and moreover may vary within the members of a variety. Useful information can be obtained by subjecting the tuber to a laboratory test for "Wart Disease", (Synchytrium endobioticum (Schilb) Percival) by which means varieties can be grouped into at least two distinct classes (6, 32). Given favourable conditions the time required to carry out this test during the winter is at least fourteen days. Another method of distinguishing potato varieties depends on the rapidity of development and the colour of the sprout. This method has the disadvantage of being somewhat lengthy especially during the "rest" period of the tuber. It will be observed that the methods given above for the differentiation of potato varieties are either uncertain if used alone or laborious in technique.

The object of the present investigation was to obtain chemical tests which would enable a rapid and accurate identification of tubers to be made. The importance of such a method of identification is obvious from a consideration of the following points. Under the regulations of the Ministry of Agriculture and the Department of Agriculture for Scotland varieties of potatoes used for seed purposes have to reach a certain standard of purity and the growing crops are inspected for this purpose. After the crops have been harvested however, confusion may arise from the similarity in appearance of the tubers of different varieties and a rapid and certain method of discrimination would in such cases be very valuable. Work of this nature has already been carried out by McIntosh (34) whose investigation although of a preliminary nature, made clear the possibility of differentiating potato varieties by chemical reactions.

Previous Work.

That intervarietal differences of a chemical nature exist between tubers of potato varieties has long been recognised. During the war an extensive study of the chemical composition of potato tubers was undertaken by a Committee of the Royal Society (43). The dry matter and total nitrogen contents of several varieties from a number of widely separated sources were determined. The results indicated that the

locality in which the plants were grown had an important influence on the composition of the tubers. It was found that the total nitrogen content of the plants grown in the North was greater than in those grown in the South; little difference existed between those from East and West. With regard to dry matter content the values for the eastern samples were greater than those of the western; samples from the north and south were very similar.

Willaman and West (50) working with American varieties found that the composition of potato tubers was not affected by varietal differences, so much as by environmental effects. Early maturing varieties, however, had a lower percentage of dry matter and higher percentages of ether extract, mineral matter, and nitrogen than later maturing varieties.

The distribution of dry matter and nitrogen was investigated by Coudon and Boussard (12) and later by Glynne and Jackson (15), who observed a greater percentage of dry matter in the cortex than in the medulla. The reverse was found for the nitrogen content.

Intervarietal differences in starch content were obtained by Johnson and Boyle (27,28) who also found that for any one variety there was a higher percentage of starch in the cortex than in the medulla of a tuber. Locality and environment also influenced the value to a fairly large extent.

It would therefore appear that the fluctuations in total nitrogen, dry matter, starch or moisture contents due to environment, render their determination unsatisfactory for the differentiation of varieties.

Investigations carried out by Sperling (45) and by Dix(13) also showed the existence of varietal differences in starch content. More recently a study of various properties of starch obtained from four different varieties of the potato was made by Kavcic (30). Varietal differences were obtained in mean diameter of the grains, content of phosphoric acid, water content in the air dried state, ash content, swelling temperature, viscosity, capacity for iodine adsorption, optical rotatory power, aggregate weight, and fraction diffusible through a collodion membrane. The methods employed were not suitable, however, for rapid routine work.

The solanine content of tubers was investigated by Artschwager (2) but substantial variations were not observed. A similar investigation was carried out by Bomer and Mattis (5) who found that varietal differences in solanin existed but that the value for the tubers of any one variety varied according to age, size and exposure to light.

There are many other substances present in the potato tuber which can be detected and estimated by chemical reactions.

For instance it has been shown that the potato tuber contains a number of different classes of

enzymes (47). Mention may be made of diatase, invertase, and pectase of the carbonydrases, and tyrosinase, oxidase and peroxidase of the oxidising enzymes. A variation in diastatic power of potato juice according to the variety of potato, was observed by Joszt and Starezewski (29). Such a method necessarily involved preliminary extraction and preparation of the plant sap and is therefore unsuitable as a means of rapid estimation.

A considerable amount of work has been published on the detection and estimation of the oxidising enzymes by means of colour reactions. This work has been reviewed at some length by Raper (40). Since such tests have been applied directly to plant tissue, it was decided to make further investigation along this line.

Several colour tests have been employed for the detection of pectic substances in plant tissue (18) and it was considered advisable to apply those to the potato.

Although some work has been carried out on the determination of hydrogen ion concentration of potato sap (4,7,24,48), no attempt seems to have been made to differentiate varieties by this means. It is possible that varietal differences do exist as is the case with apples (22), and since a rapid and accurate determination of hydrogen ion concentration is comparatively easy to make it was decided to collect

data on the subject.

The analysis of potato tubers by various workers reveals the presence of amino acids and phenols (46). Both those groups of substances lend themselves to detection through the formation of coloured dyes; and it therefore seemed advisable to examine the possibility of varietal differences in the potato by such methods.

Preliminary Investigations.

In order to obtain some idea of the applicability of the colour reactions used by McIntosh, the following tests were repeated:-

1. The Alkali Test.
2. The Oxidase Test.
3. Tests for Tyrosinase (a) Blackening of Potato Tissue
   (b) Tyrosinase p.Cresol Reaction.

1. The Alkali Test. When a section of potato tuber is immersed in a strongly alkaline solution such as normal sodium, potassium, or ammonium hydroxides, a yellow colour develops and remains constant after approximately five minutes. A summary of results is given in Table 1.

The yellow colour is attributed by McIntosh to "flavones". This is doubtful however since standard reactions for the flavone group (17,37) and the test described by Shibata, Nagai and Kishida (44) gave negative results when applied to untreated tissue and

Table 1. The Alkali Test.

Colours obtained with Normal Potassium Hydroxide Solution.

Light Yellow	Yellow	Dark Yellow
Eclipse	Bishop	Abundance
Edzell Blue	British Queen	Ally
Puritan	Crusader	Arran Chief
Witchhill	Epicure	Arran Comrade
	Golden Wonder	Arran Victory
	Katie Glover	Champion
	King Edward	Great Scot
	Langworthy	King George
	Ninetyfold	Lochar
	President	Majestic
	Rhoderick Dhu	Tinwald Perfection
	Up to Date	

and to alcoholic tissue extracts. In any case small differences in the intensity of the colour obtained with alkalies are difficult to estimate. Furthermore the yellow colour of the flesh of certain varieties of potatoes, varies with the source of the tubers. This test was therefore regarded as being unlikely to yield favourable results.

2. The Oxidase Test. The presence of an oxidase in the potato may be demonstrated by the catalytic oxidation of such substances as benzidine and the leuco base of malachite green. When an alcoholic solution of benzidine is applied directly to a section of potato tuber a brown-purple colour is produced. Table 2, gives a summary of results obtained with a 0.5% solution of benzidine in 50% ethyl alcohol. The colour produced was not uniform throughout the section but appeared to concentrate round the cortex, vascular bundles and small injuries, giving the section a patchy appearance. Moreover the brown purple formed is not a colour which lends itself to accurate colour analysis. Accordingly a closer examination of the oxidase of the potato was postponed in favour of the tyrosinase p.cresol test described below.

3. Tests for Tyrosinase.

(a) The Blackening of Potato Tissue.

When a potato tuber is cut and exposed to the air a reddish brown colour appears, which is later replaced by black. The reactions are brought about

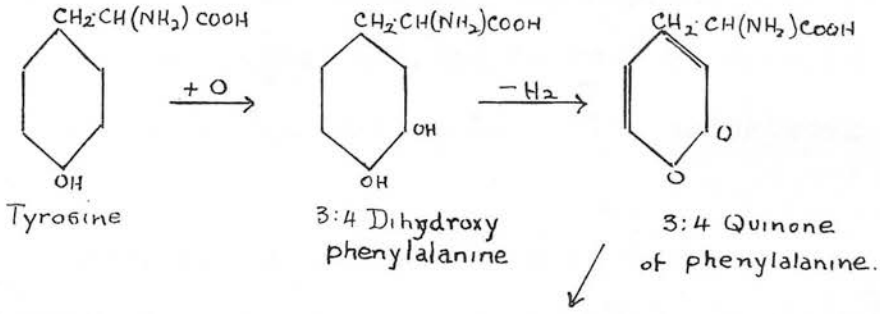
Table 2. The Oxidase Test.

Colours obtained with a solution of 0.5% benzidine in 50% alcohol.

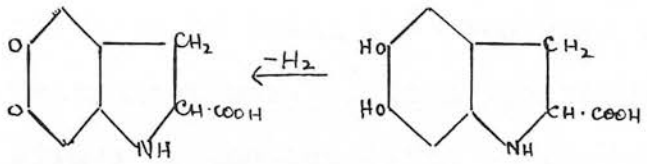
Dark Brown Purple	Intermediate	Light Purple.
Abundance	Ally	Eclipse
Bishop	Arran Chief	Edinburgh Castle
Edzell Blue	Arran Comrade	Epicure
Fortyfold	Arran Victory	Dunotter Castle
Katie Glover	Dargill Early	Harbinger
King George	Great Scot	King Edward
Lochar	May Queen	Puritan
Majestic	Nintyfold	
President	Rhoderick Dhu	
	Sharpe's Express	
	Tinwald Perfection	
	Up to Date	
	Witchhill	

30 - 40 tubers of each variety were tested.

by tyrosinase and consist in the oxidation of the naturally occurring amino acid. Raper (40) suggests that the series of reactions which take place in the oxidation are as follows:-

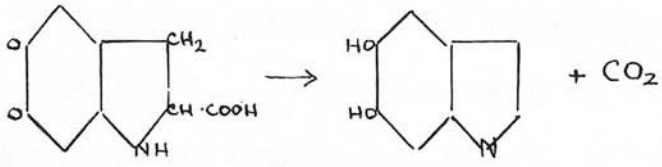


Tyrosine — 3:4 Dihydroxy phenylalanine — 3:4 Quinone of phenylalanine.



5:6 Quinone of Dihydroindole - 2 carboxylic acid. 5:6 Dihydroxidihydroindole - 2 carboxylic acid.

The 5:6 quinone of dihyroindole 2 carboxylic acid is believed to be the red substance which is the first visible product of the enzyme action. The enzyme is not necessary for the two further stages of the reaction the first of which is the formation of a colourless product by intramolecular change.



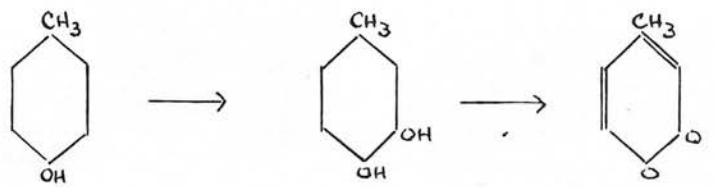
Red Quinone — 5:6 Dihydroxy-Indole.

The final stage of the reaction is the oxidation of 5:6 dihydroxyindole to melanin.

A study of the kinetics of the tyrosinase-tyrosine reaction made by Raper and Wormall (41) and Haehn and Stern (21) showed that it was of the monomolecular type. The last named workers found that the coefficient of the reaction was dependent on the concentration of enzyme and not on that of tyrosine. The optimum p.H of tyrosinase was given as between pH6 and pH8 (41).

The formation of melanin in a section of potato tuber depends on the variety (34). A similar result was obtained by Haehn (20) who used extracted sap in his determinations. In a preliminary experiment slices of the tubers to be tested were treated with a solution of sodium carbonate (pH about 8) and laid out on a glass plate in a warm atmosphere. The colour was very slow in appearing and tended to concentrate at irregularities on the surface of the tissue giving the section an uneven appearance. An example of the results is given in Table 3.

(b) Tyrosinase p.Cresol Reaction. Besides acting on tyrosine, tyrosinase also brings about the oxidation of certain monohydric phenols (8) including phenol, p.cresol and catechol. When p.cresol is used as the substrate a brilliant orange-red colour is produced. According to Pugh and Raper (39) this is due to the formation of an orthoquinone as follows:-



p-Cresol Homo catechol Homoquinone.

Table 3. Tyrosinase - Tyrosine Reaction.

Dark	Intermediate	Light
British Queen	Abundance	Dunotter Castle
Katie Glover	Ally	Golden Wonder
King George	Arran Chief	Harbinger
Majestic	Arran Victory	May Queen
	Bishop	President
	Eclipse	
	Edzell Blue	
	Great Scot	
	Nintyfold	
	Puritan	
	Rhoderick Dhu	
	Tinwald Perfection	
	Up to Date	
	Witchhill.	

It appears likely that this formation (homoquinone) is only the first part of the reaction and that higher products of unknown constitution are produced.

A dilute aqueous solution (0.2%) of p.cresol was used to treat the sections of potato tuber and an orange-red colour, which in most cases was uniform throughout the section, appeared in about 5-10 minutes. A summary of results is given in Table 4.

A colour analysis of the orange-red tint showed that it was composed of red and yellow in the approximate ratio of 2 to 1.

The estimation of this type of colour is comparatively simple, since only two primary colours are used. The measurement of a brown or brown purple such as is produced in the oxidase test is obtained by a combination of the three primary colours, and requires more time and manipulative skill.

The ease with which this reaction can be applied, and the uniformity and nature of the colour produced made the test highly suitable for the present investigation; it was therefore decided to study the tyrosinase p-cresol reaction in more detail.

During the preliminary investigations it was clearly indicated that improvement and standardisation of laboratory technique were essential. In the first place the varietal differences obtained by the methods already described, were relative, and some means had to be devised whereby an absolute value

Table 4. Tyrosinase - p.Cresol Reaction.

0.2% Solution of p.Cresol

Dark Red	Intermediate	Light Orange
Bishop	Abundance	Arran Victory
British Queen	Ally	Dunotter Castle
Crusader	Arran Comrade	Eclipse
Edzell Blue	Arran Chief	Kerr's Pink
Golden Wonder	Epicure	King Edward
Immune Ashleaf	Great Scot	
Katie Glover	Harbinger	
King George	Rhoderick Dhu	
Majestic	Tinwald Perfection	
President	Up to Date	

30 - 40 tubers of each variety were tested.

of the colour which developed could be ascertained. A tintometer was therefore constructed by means of which the intensity of the colour produced on a tuber section was compared with a colour standard.

TYROSINASE p.CRESOL REACTION.

(1) Laboratory Technique

(a) Description of Tintometer and Estimation of Colour.

(b) Kinetics of the Reaction.

(2) Factors affecting the Reaction.

(a) Maturity

(b) Environment

(c) Variety

(d) Disease etc.

(e) Season

(f) Storage

(3) Discussion of Results.

(4) Conclusions.

(1). Laboratory Technique.

(a) Description of Tintometer and Estimation of Colour.

A diagram with dimensions of the tintometer is given in Fig.1. The instrument consisted of a narrow cardboard box, A, tapering at the top end, in which an eyepiece B was fixed. At a short distance (7.5 cms.) from the top was placed a sheet of cardboard C, containing two apertures cd and c'd'; a plan of this is given in Fig.2. Below the aperture cd a rectangular slot D was cut in the front of the box. The purpose of this slot was to enable a colour

DIAGRAM OF TINTOMETER.

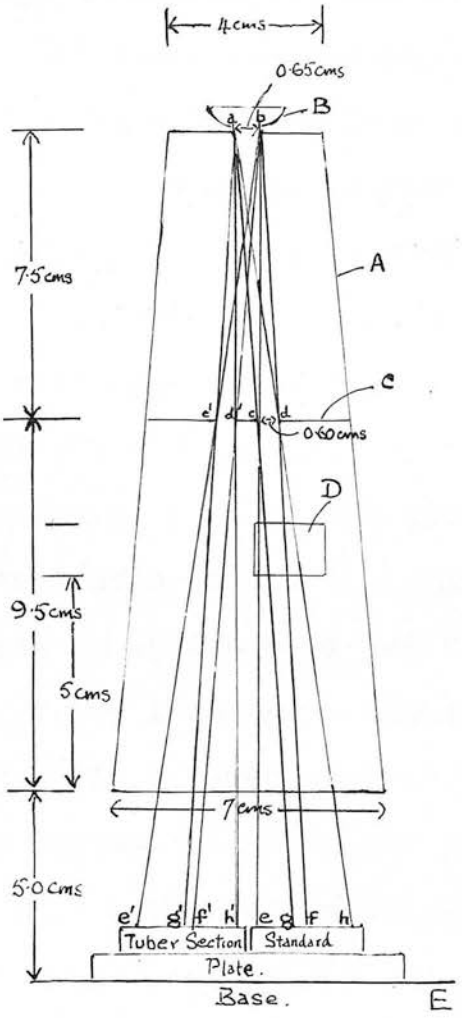
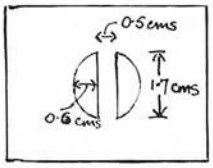


Fig 1



Plan of C

Fig 2

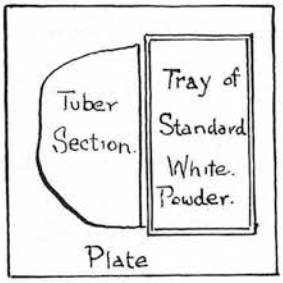


Fig. 3.

standard to be placed in the path of the rays passing through cd to the lens ab in the eyepiece. A small cardboard rest in the back of the tintometer opposite D served as a rest for the colour standard. The whole instrument was suspended at a calculated distance above the base E. The lens a.b. had an aperture of 0.65 cms. and a focal length of 7.5 cms. This is to say that when the eyepiece was in position at the top of the box, the apertures cd and c'd' were at the focal distance from the lens. Rays passing from a through c and d impinged on the base at g and h respectively, whilst rays passing from b through c and d arrived at e and f. Hence any object within the area eh on the base was visible although not in focus at the lens ab. Similarly rays from e'h' through c'd' arrived at ab. Any slight irregularities of the surface of an object placed within the spaces eh or e'h' merge and an average intensity is obtained at the eyepiece. Since the rays pass through the aperture at cd or c'd' the edges of which are in focus the result is a sharply defined area of even illumination. The colour values were obtained by the use of Lovibond's Colour Standards in conjunction with a standard white background, the source of light being a Dakol daylight lamp. The colour standards consisted of a series of red, yellow and blue glasses of varying intensity, and by combining these, any colour could be matched. The intensities of the colour standards are given numerically. The base of the instrument

rested on an electric hot plate, employed to control the temperature. To obtain greater uniformity of temperature and illumination, the apparatus was surrounded by a cardboard box containing apertures for the lens and thermometers, and to permit of the manipulation of the colour standards.

The procedure adopted was as follows. A section about 0.75 cms. thick was cut at right angles to the long axis of the potato tuber and covered with 2 or 3 drops of reagent after which it was allowed to stand on filter paper for 30 seconds to drain. By this method all sections, irrespective of size, received the same amount of reagent per unit area. A small piece was then cut from one side of the section, which then appeared roughly semicircular. The treated section was placed on a porcelain plate alongside a tray containing the standard white background as in Fig.3. The plate and its contents were than set on the base of the instrument so that the tuber section and standard white appeared in the areas e'n' and e n respectively. The colour of the section was matched by means of standard colour glasses placed in the slot D. In this way the colour of the section was estimated at definite intervals of time after treatment with reagent, and a graph of colour intensity against time was obtained.

(b) Kinetics of the Reaction

This part of the investigation was divided into three parts.

(i) Substrate Concentration and Velocity of Colour Formation.

(ii) Velocity of Reaction

(iii) Temperature Coefficient of the Reaction.

i. Substrate Concentration and Velocity of Colour Formation.

When the 0.2% solution of p.cresol was used as in the preliminary experiment already described, the orange-red oxidation product was slow in developing, and in order to speed up the reaction it was decided to make use of a more concentrated substrate. A very concentrated aqueous solution could not be obtained since the solubility of p.cresol is 1.9% at $20^{\circ}C$. Two other solvents were used, namely, ethyl alcohol and sodium hydroxide solution, in both of which p.cresol is easily soluble.

Ethyl Alcohol as Solvent. Solutions containing .5%, 1%, 2%, 4% and 8% p.cresol in absolute ethyl alcohol were prepared. These were used with the variety Arran Comrade, and the colour produced was measured with the aid of the tintometer. The intensity of the red component of the orange colour was plotted against time (Graph 1). The values for the yellow component were not analysed; a reason for this will be given in paragraph ii. Results given in

GRAPH 1

GRAPHS OF COLOUR AGAINST TIME

p CRESOL SOLUTIONS IN ALCOHOL.

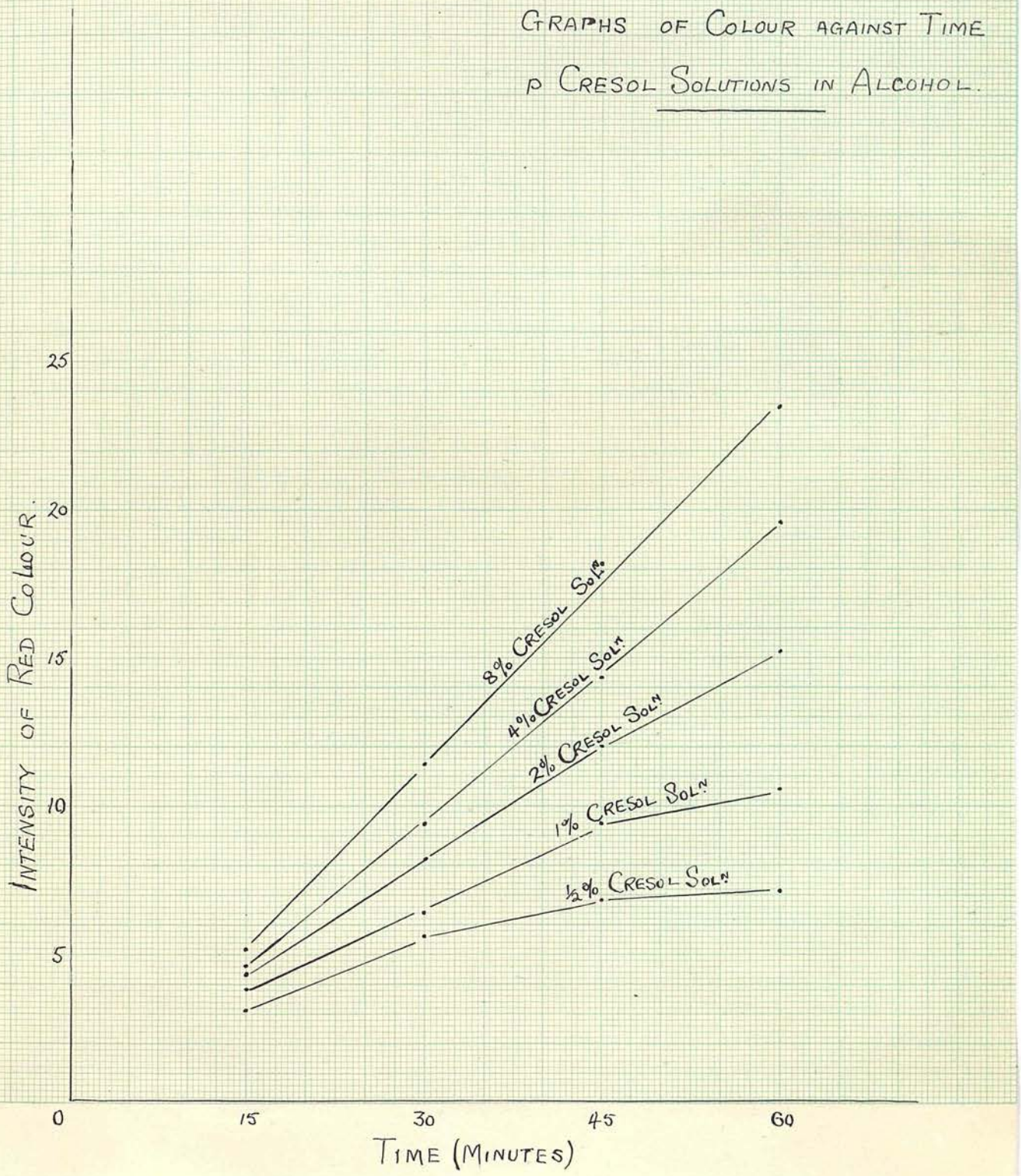


Table 5. Substrate Concentration and Velocity of Colour Formation using Ethyl Alcohol as Solvent.

Intensity of Colour given in Lovibond's Colour Units.

Time Interval	0.5% p.cresol		1.0% p.cresol		2% p.cresol		4% p.cresol		8% p.cresol	
	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow
15 mins.	3.1	2.5	3.8	3.0	4.3	3.3	4.6	3.6	5.2	4.6
30 "	5.6	4.2	6.4	4.6	8.2	5.4	9.4	6.0	11.4	6.8
45 "	6.8	4.2	9.4	5.4	12.0	6.6	14.3	7.4	18.2	8.0
60 "	7.1	4.6	10.6	5.4	15.2	7.2	19.6	7.8	23.5	9.0

See Graph 1.

Table 5 indicated that the velocity of colour formation was increased with an increase in substrate concentration. The alcohol solvent, however, evaporated on the tuber section, leaving a dried surface on which the colour was often irregular. Furthermore it should be noted that alcohol is frequently employed to precipitate oxidising enzymes in plant tissue, so that the course of the tyrosinase p.cresol reaction may be influenced by the alcohol present.

Sodium Hydroxide Solution as Solvent. Solutions of p.cresol in sodium hydroxide solutions were prepared as follows:-

0.5%,1%,2% and 4% p.cresol in a solution containing the equivalent quantity of NaOH.

" " " " " " " half the " " "

" " " " " " " quarter the " " "

The equivalent quantity of NaOH was calculated from the equation

$$C_6H_4.CH_3.OH + NaOH \longrightarrow C_6H_4.CH_3.ONa + H_2O$$

p.cresol — Sosium salt of p cresol.

Results obtained with those solutions from the variety Arran Comrade are given in Tables 6 and Graph 2.

The most suitable solution was that containing 2% cresol in $\frac{1}{2}$equivalent NaOH. The graph of intensity of red colour against time for this solution had a comparatively long linear period from which the

GRAPH 2

GRAPHS OF COLOUR AGAINST TIME
p CRESOL SOLUTIONS IN NaOH.

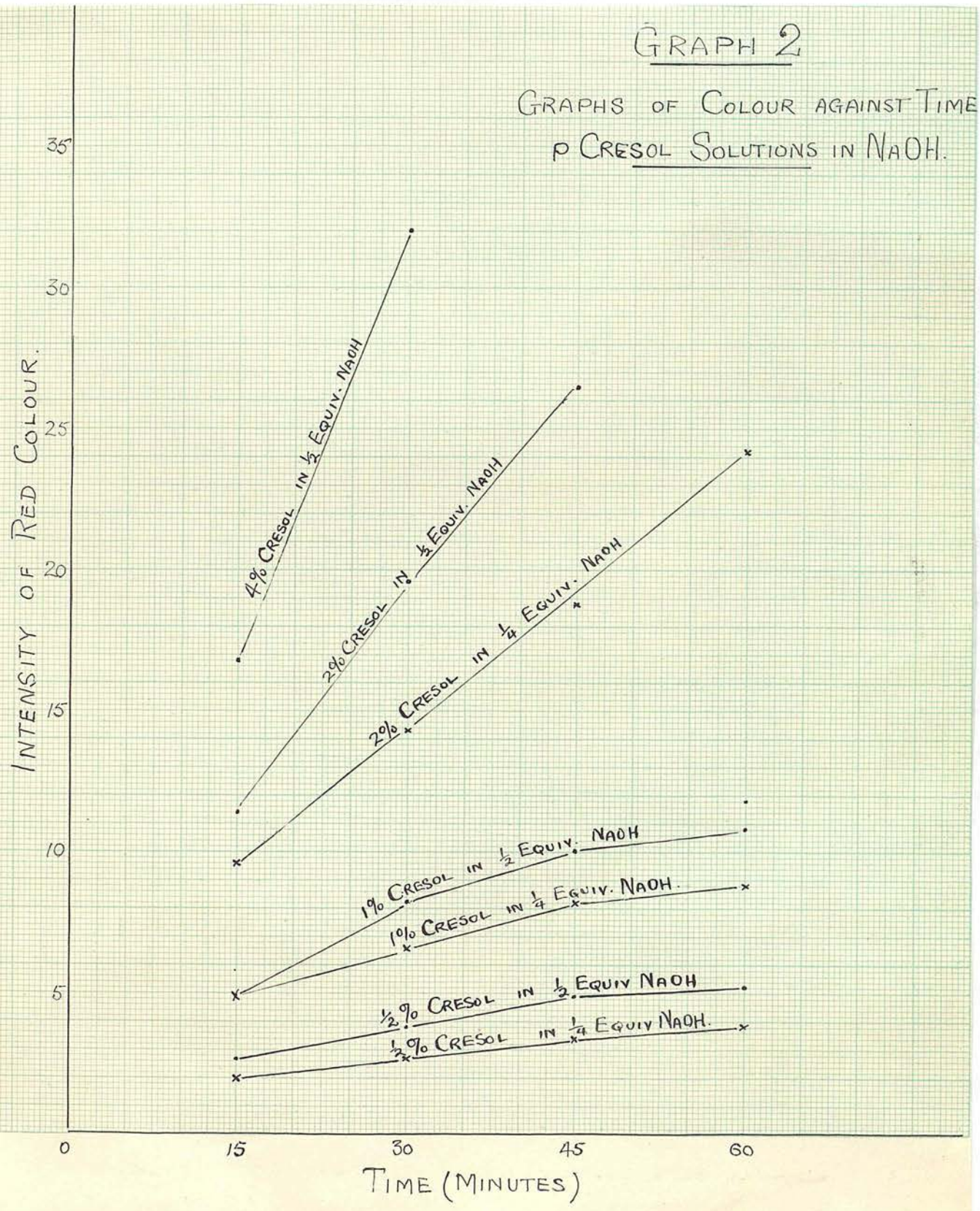


Table 6. Substrate Concentration and Velocity of Colour Formation using Sodium Hydroxide Solution as Solvent.

The Intensity of Colour produced with p-Cresol Solutions containing the equivalent quantity of NaOH was very difficult to estimate. After 15 minutes the cortex appeared bright red whilst the medulla remained light yellow. After an hour the section had assumed a very irregular and patchy appearance.

Intensity of Colour produced with p.cresol Solutions containing ½ the equivalent quantity of NaOH. (Intensities are in Lovibond's Colour Units).

Time Interval	0.5% p.Cresol		1.0% p.Cresol		2.0% p.Cresol		4.0% p.Cresol	
	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow
15 mins.	2.7	2.5	4.9	3.6	11.4	5.4	16.8	6.0
30 "	3.8	3.0	8.2	4.2	19.6	5.8	32	6.6
45 "	4.9	3.3	10.0	4.6	26.5	6.0	-	-
60 "	5.2	3.3	10.8	5.4	-	-	-	-

Intensity of Colour produced with p.Cresol Solutions containing ¼ the equivalent quantity of NaOH.
(Intensities are in Lovibond's Colour Units.)

Time Interval	0.5% p.Cresol		1.0% p.Cresol		2.0% p.Cresol	
	Red	Yellow	Red	Yellow	Red	Yellow
15 min.	2.0	2.5	4.9	3.6	9.6	5.0
30 "	2.7	3.0	6.6	4.2	14.3	6.0
45 "	3.4	3.0	8.2	5.0	18.8	6.8
60 "	3.8	3.0	8.8	5.4	24.2	7.2

See graph 2.

average velocity constant for the formation of red colour was calculated according to the formula

$$k = \frac{dr}{dt}$$

where k is the velocity constant of the red colour reaction

r is the intensity of the red colour produced in time t.

It was decided to employ this solution thereafter and to obtain a constant for each variety from the linear portion of the graphs.

It will be noticed that a 2.0% solution of p.cresol in ½ equivalent NaOH was more effective than an 8% alcoholic solution. It is possible that the alkaline solution provided a substrate with a pH close to the optimum reaction for tyrosinase (pH 8); the alcoholic solution was of course acid (pH 5).

ii. Reaction Velocity of Tyrosinase p.Cresol Reaction.

As has been mentioned previously the reaction produced an orange-red decomposition product, which was represented by a combination of red and yellow standard colours. It was necessary to know whether both colours proceed from the same reaction, in which case a study of either would yield sufficient information to determine the reaction velocity.

In order to prove whether or not this was the case, sections of tubers of a number of varieties were treated with p.cresol solutions in ½ equivalent NaOH, 0.5% and 1% Cresol solutions were used so that the final colour was not too dense for measurement.

The colours produced were estimated at fixed intervals of time until the reaction had ceased. The temperature remained constant. Values of both red and yellow colour intensities were plotted against time and graphs obtained. An example is given in Table 7 and Graph 3. Further results are included in the Appendix p.64,65. In both cases the curves consisted of a short linear period at the commencement, but as the reaction proceeded it became logarithmic. The red colour was considered first.

If the final intensity of this (r_∞) is taken as being identical with the initial concentration of substrate, then the amount of unchanged substrate after time t is given by the difference of r_∞ and the intensity of the red colour at time t (r_t).

By plotting $\log \frac{\text{Initial Substrate concentration}}{\text{Substrate concentration after time t.}}$ (i.e. $\log \frac{r_\infty}{r_\infty - r_t}$) against time a straight line graph was obtained, showing that the course of the reaction followed the monomolecular law.

Values of the velocity constant k_r for the reaction were obtained from the formula,

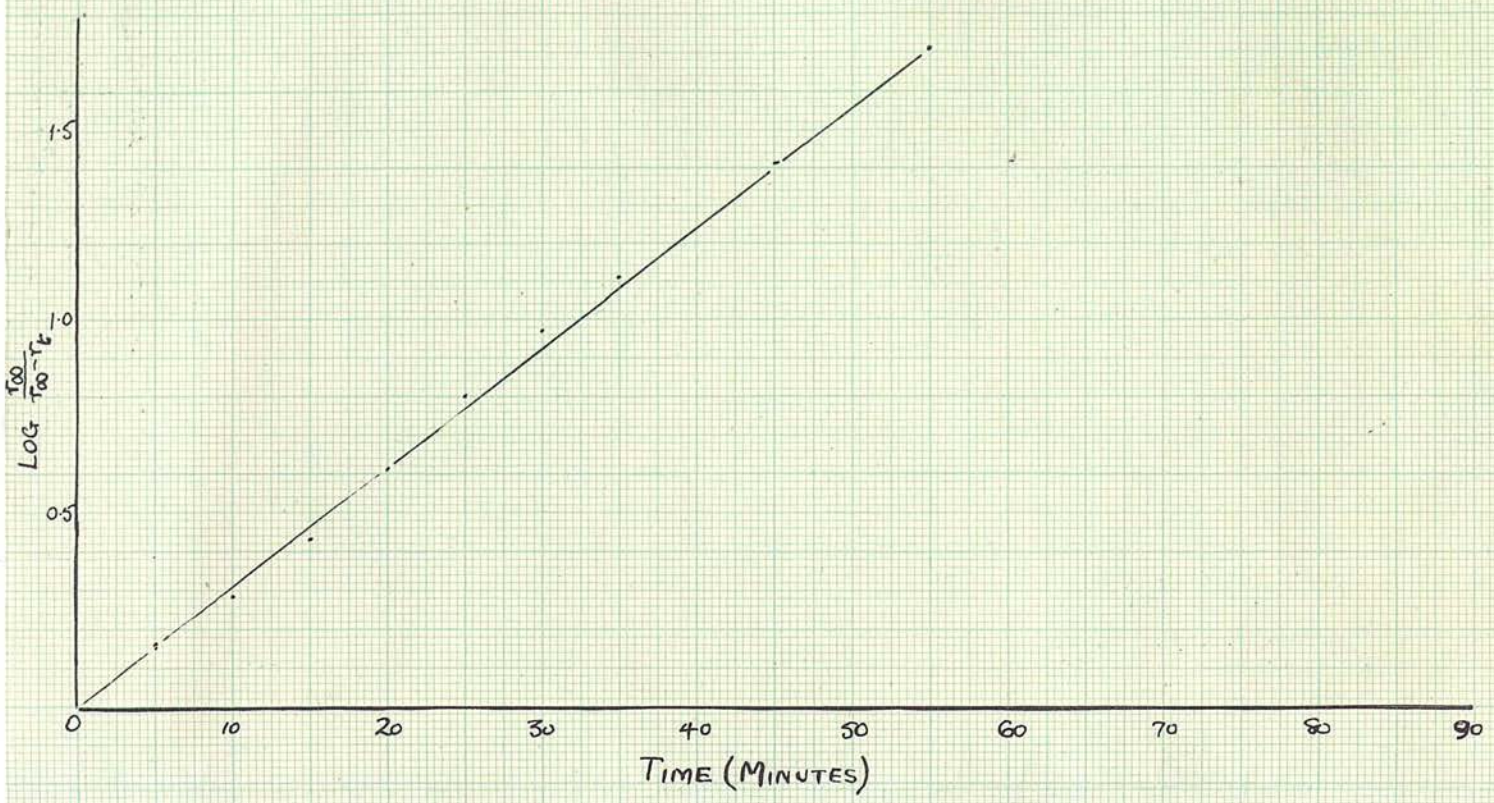
$$k_r = \frac{1}{t} \log \frac{r_\infty}{r_\infty - r_t}$$

and proved to be approximately constant throughout the reaction.

The values for the intensity of the yellow colour were treated in a similar manner. A correction for the original yellow colour of the section was

GRAPH 3.

GRAPH OF LOG. $\frac{T_\infty}{T_\infty - T_t}$ AGAINST TIME



GRAPHS OF COLOUR AGAINST TIME

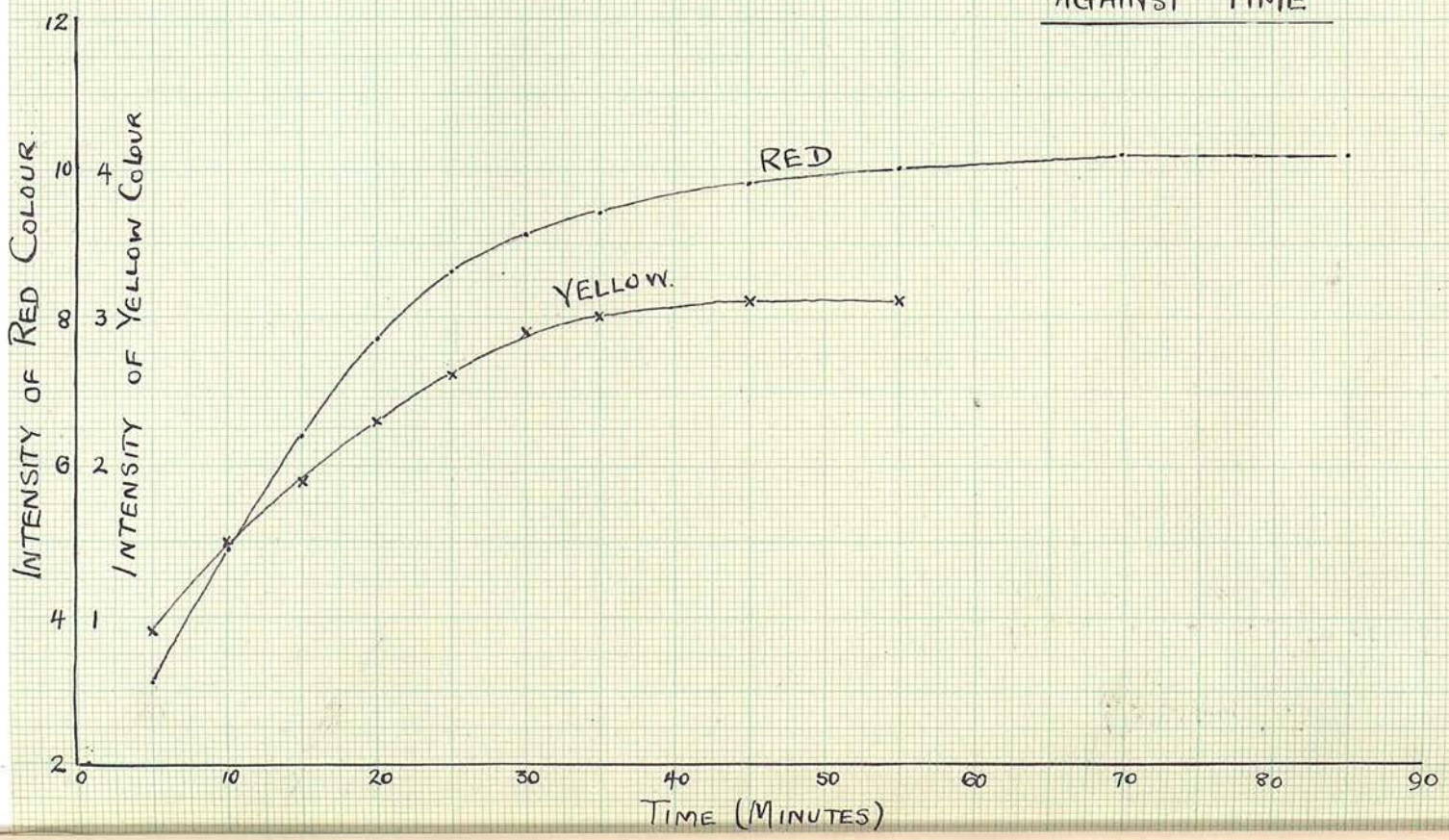


Table 7. Reaction Velocity of Tyrosinase p.Cresol Reaction.

Colour Intensity produced on a section of the variety "President" with 0.5% solution of p.cresol in $\frac{1}{2}$ equivalent NaOH. Temperature 18°. Intensities are given in Lovibond's Colour Units.

Time Interval t	Intensity of Red Colour	Intensity of Yellow Colour		$\log \frac{r_\infty}{r_\infty - r_t}$	$\log \frac{y_\infty}{y_\infty - y_t}$	kr	ky
		Observed	Corrected				
0 min.	-	2.7	-	-	-	-	-
5 "	3.1	3.6	0.9	0.16	0.15	0.072	0.069
10 "	4.9	4.2	1.5	0.28	0.29	0.066	0.067
15 "	6.4	4.6	1.9	0.43	0.41	0.066	0.063
20 "	7.7	5.0	2.3	0.61	0.59	0.070	0.068
25 "	8.6	5.4	2.7	0.80	0.89	0.074	0.082
30 "	9.1	5.5	2.8	0.97	1.01	0.074	0.077
35 "	9.4	5.7	3.0	1.11	1.49	0.073	0.098
45 "	9.8	5.8	3.1 = y_∞	1.41	-	0.070	-
55 "	10.0	5.8	3.1	1.71	-	0.071	-
70 "	10.2 = r_∞	5.8	3.1	-	-	-	-
85 "	10.2	5.8	3.1	-	-	-	-

See Graph 3.

deducted from each reading, and the velocity constant k_y was obtained from the corrected readings.

On comparison it was found that the two constants k_r and k_y were in close agreement, indicating that the red and yellow colours proceed from the same reaction. Hence a study of the development of one of the colours provided sufficient information to determine the velocity of reaction. The observation of the red colour possessed several advantages as it was formed more rapidly and was more easily measured than the yellow colour. Moreover potato tubers possess a light yellow flesh, a factor which is variable, and a correction had to be applied for this each time. Accordingly the course of the tyrosinase-p.cresol reaction was subsequently followed by analysing the formation of the red colour.

iii. Temperature Coefficient of the Reaction.

It was recognised that the velocity of reaction would increase with a rise of temperature, and in order to obtain some idea of the magnitude of such a change, a series of readings for the rate of reaction were obtained for a range of temperatures extending from $8^{\circ}C$ to $28^{\circ}C$. The reagent used was a 2% solution of p.cresol with $\frac{1}{2}$ equivalent quantity of NaOH. When the intensities of the red colour were plotted against time, linear graphs were obtained from which the velocity constants for the formation of red

colour were calculated. The temperature coefficient of the reaction, which shows the relation of the reaction velocity (k_t) at temperature t, and at a temperature 10° higher, that is $\frac{k_t + 10}{k_t}$, was calculated. An example is given in Table 8 and Graph 4, while further examples are included in the appendix (p.66). The results show that with a temperature increase of 10°C the increase in reaction velocity is approximately twofold.

Under existing laboratory conditions it was found that the temperature within the tintometer box could be most easily maintained at 18°C., hence this temperature was taken as a standard for subsequent observations.

The standardised laboratory technique was as follows. A section of potato tuber was treated with a few drops of 2% p cresol solution containing ½ equivalent amount sodium hydroxide and allowed to drain for 30 seconds. It was then placed in the tintometer at a temperature of 18°C and the colour estimated at 3 minute intervals for a period of 15 minutes. Colour estimations were discontinued in most cases after the section assumed an intensity of red colour greater than 20 Lovibond's Colour Units. A graph of red colour intensity (r) against time (t) was obtained and from this a reaction constant k was calculated

$$k = \frac{dr}{dt.}$$

The slope of the graph represented by k did not

GRAPH 4

RELATION BETWEEN TYROSINASE - p CRESOL REACTION AND TEMPERATURE

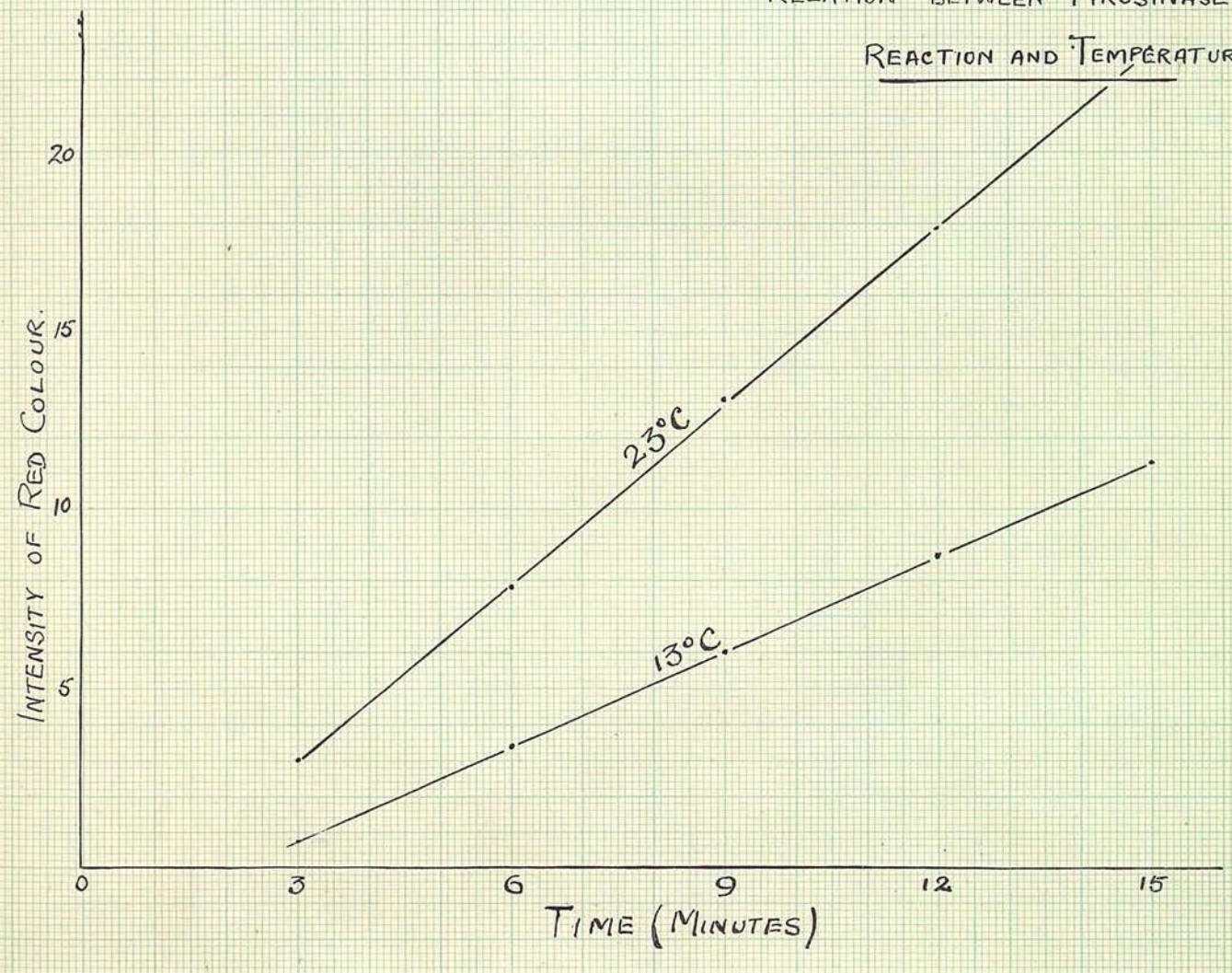


Table 8. Temperature Coefficient of the Tyrosinase p.Cresol Reaction.

Colour Intensities (Lovibond's Colour Units) produced with variety Rhoderick Dhu.

Reagent - 2% p.cresol with $\frac{1}{2}$ equivalent NaOH.

Time Interval	Temperature 13°C.		Temperature 23°C.	
	Red	Yellow	Red	Yellow
3	less than 2.0	3.4	3.0	3.4
6	3.4	3.6	7.8	4.2
9	6.0	4.1	13.1	5.5
12	8.7	4.6	17.9	6.6
15	11.3	5.0	22.9	6.8

The values for Colour Intensities are averages of four determinations.

From Graph 4 is obtained $k_{13} = 0.88$

$k_{23} = 1.66$

The temperature coefficient $\frac{k_{23}}{k_{13}} = 1.89.$

necessarily determine the course of the reaction, because the graphs obtained from different varieties had different intercepts on the r axis. The reason for this is not clear but it was possibly due to complications in the initial stages of the reaction. In order to determinate the course of the reaction for any one variety the slope of the graph (k) has been supplemented by the value of the red colour at time 10 minutes (r_{10}). **Estimates of the standard error are given in the appendix p.70.**

(2) Factors Affecting the Reaction.

(a) Maturity of the Potato Tuber. In order to examine the effect of maturity upon the reaction, it was arranged to follow the growth of a number of plants throughout the summer of 1929. For this purpose six well known varieties were chosen namely, Duke of York, Epicure, Great Scot, Golden Wonder, Majestic and Ally. Those varieties differ widely in time of maturing, Duke of York and Epicure being in the "First Early" class, Great Scot, Majestic and Ally in the "Early Maincrop", and Golden Wonder in the "Late Maincrop" groups. The potatoes were grown in Midlothian at seven different localities referred to as A,B,C etc., within a radius of about six miles. At an early stage of growth two healthy and characteristic plants were selected and marked for examination. The following table gives the variety marked at the different centres.

Duke of York	Epicure	Great Scot	Golden Wonder	Majestic	Ally
A	A	A	A	A	A
B	-	B	-	-	-
C	C	C	C	C	C
D	D	D	D	D	D
-	E	-	-	-	E
F	-	-	-	F	-
-	G	G	-	-	-

A - College Gardens, Liberton, 2½ miles S.

B - College Farm, Bogmall, 5½ " ".

C - Dept. of Agriculture Seed Testing Station, East Craigs, Corstorphine, 5 miles W.

D - Messrs. Dobbie & Co., Joppa, 4 miles E.

E - J.R.Gray, Esq., Southfield, Duddingston, 2½ miles E.

F - R.J.M.Brockley, Esq., Gorebridge, 10 miles S.E.

G - R.L.Scarlett, Esq., Inveresk, 6½ miles E.

At intervals up to complete ripening a few tubers were taken from each plant. The samples were taken to the laboratory as quickly as possible where they were weighed and treated with the standard cresol reagent. (2% p.cresol with ½ equivalent NaOH), and the velocity constant for the reaction estimated as previously described. The temperature was maintained at 18°C. An example of the results is given in Table 9 and graph 5. The complete table of results is given in the Appendix p. 67.

GRAPH 5.

RELATION BETWEEN TYROSINASE-P CRESOL REACTION AND MATURITY.

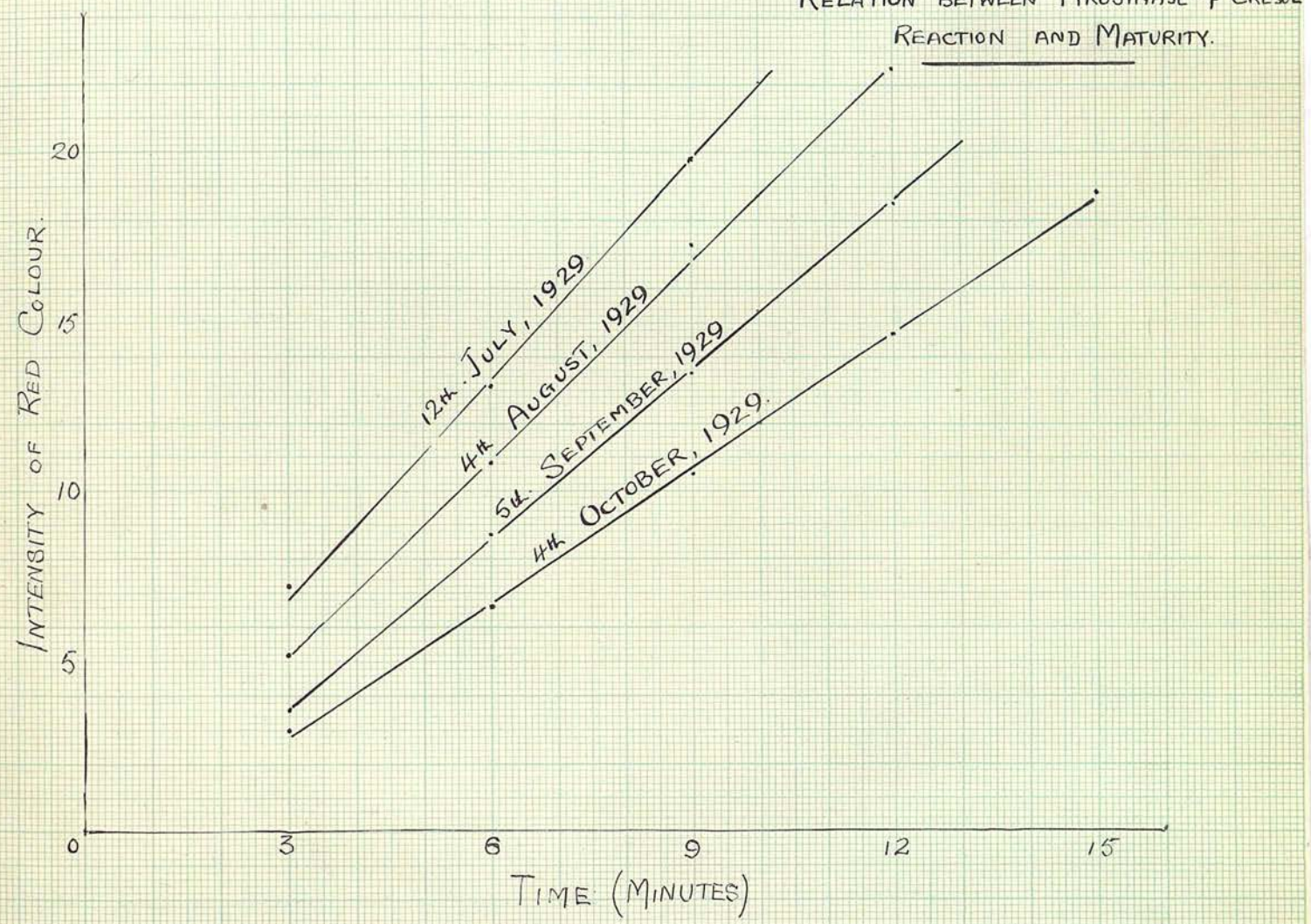


Table 9.

Relation between Tyrosinase p.Cresol Reaction and Maturity of Tuber.

Colour Intensity produced with standard p.cresol reagent (Lovibond's Colour Units).

Variety - Majestic. Source - C.

Time Interval	12th July, 1929.		4th August, 1929		5th September, 1929		4th October, 1929	
	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow.
3 min.	7.2	3.3	5.2	2.8	3.6	2.5	3.0	3.6
6 "	13.1	4.6	10.8	4.1	8.7	4.5	6.6	4.6
9 "	19.8	5.6	17.3	4.8	13.5	5.2	10.5	5.4
12 "	-	-	22.5	5.3	18.5	5.8	14.7	6.0
15 "	-	-	-	-	-	-	18.8	6.6
k	2.2	-	1.9	-	1.7	-	1.3	-
r_{10}	22.00	-	18.7	-	15.3	-	12.0	-

The figures given above represent the average of four values obtained from duplicate tubers from each of two plants.

The values for k and r were obtained from graph 5.

An examination of the data indicated that the activity of potato tyrosinase varied with the age of the tuber, being greatest at the early stages of tuberisation and gradually diminishing as the plant matured. With the six varieties examined a minimum reactivity was usually observed a short time before complete maturity was reached, and the occurrence of this period depended on the source of the tubers. At the same stage of maturity the size of the tuber had no influence on the enzyme activity. A few typical examples are given in Table 10 (a) and (b). The values given in table (b) were obtained from the analyses of "second growth" tubers. During the latter part of July 1929 a spell of exceptionally dry weather was experienced, which caused a check in the growth of the potato plants. This was followed in August by an abundant rainfall which brought about a rapid increase in the growth of the plants. When the Golden Wonder crop at D was examined in the beginning of September it was found that a large number of the tubers had formed new shoots upon which "second growth" tubers were appearing. A number of the "parent" tubers along with the "second growth" tubers were taken to the laboratory for analysis. When weighed it was found that some of the "young" tubers were heavier than the "old" tubers from which they had formed. The tyrosinase activity, however, was greatest in the "young" tubers, except in the samples D, which were obtained

Table 10.

Relation between Tyrosinase p.Cresol Reaction and weight of Tuber.

(a) Variety - Duke of York Source A.

Date of Sampling	Tuber Weight in grams.	k	r_{10}
25 . 7 .29	146	1.7	14.3
	62	1.7	13.4
4 . 9 .29	126	1.5	11.8
	27	1.5	12.1
7 .10 .29	250	1.4	12.4
	103	1.4	12.2

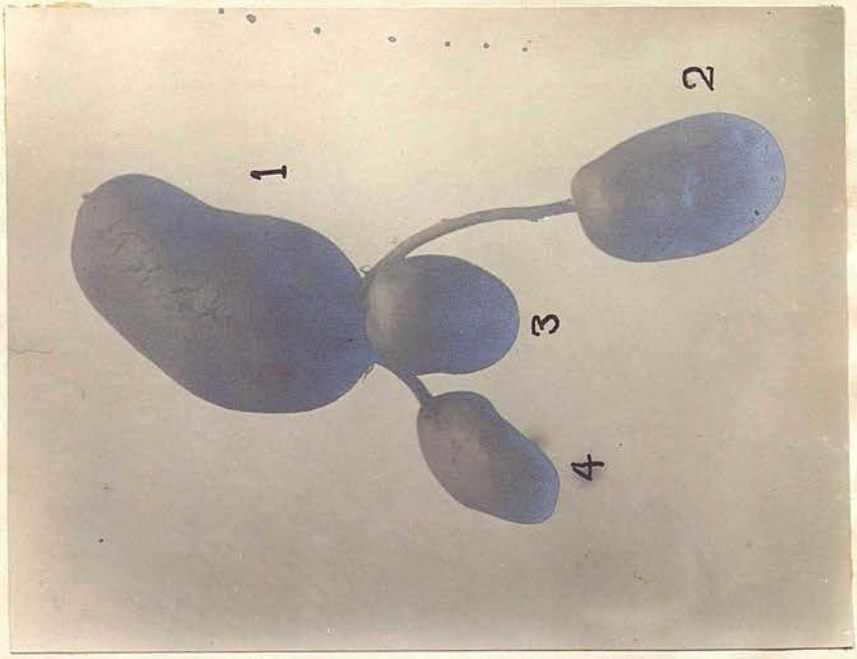
(b) Variety - Golden Wonder Source D

Date of Sampling	No.of Tuber	Tuber Weight in grams.	k	r_{10}
6. 9.29	A 1	28	1.5	13.5
	2	33	2.1	21.0
26. 9.29	B 1	92	1.3	13.5
	2	28	1.5	15.6
	3	17	1.5	16.5
	4	11	1.5	16.8
26. 9.29	C 1	50	1.3	13.3
	2	62	1.6	16.3
	3	35	1.6	16.1
4.10.29	D 1	60	1.5	14.5
	2	38	1.5	14.3

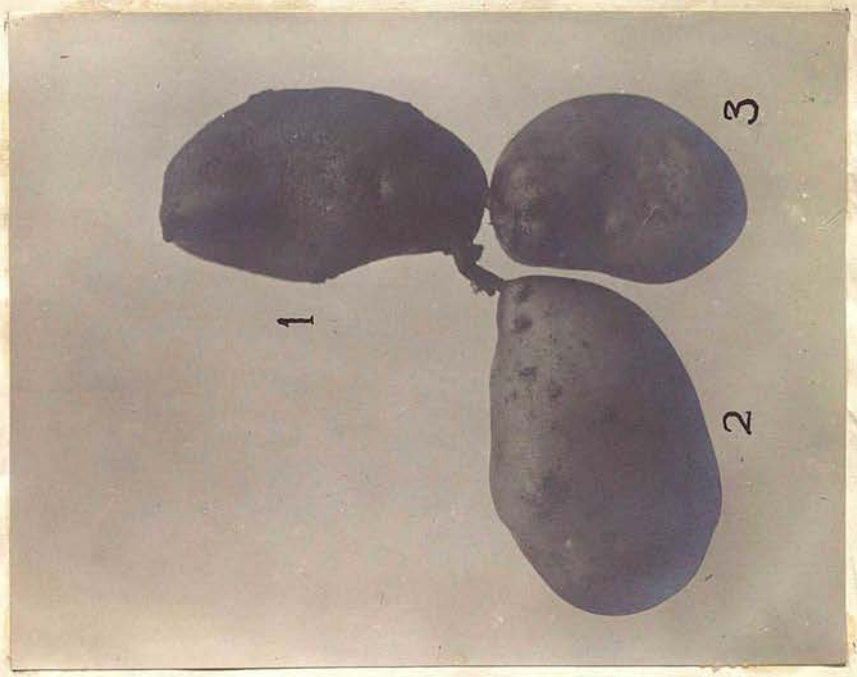
Tubers A 1; B 1; C 1 and D 1 are "parent tubers"
A 2; B 2,3,4; C 2,3; D 2 are "second growth tubers".

Relation between Tyrosinase - p.Cresol Reaction and weight of tuber. (See Table 10.).

(b) Variety - Golden Wonder Source D.



Tubers B 1,2,3 and 4.



Tubers C 1,2 and 3.

after the plant had fully matured. An examination of the results obtained for the "second growth" tubers showed that their tyrosinase activity was greatest in the early stages of growth and gradually diminished as the plant approached maturity. In the case of a sample obtained from a mature plant (D 1.2) the activity was the same in both "parent" and "second growth" tubers. These observations show conclusively that tyrosinase activity is related to stage of maturity and not to weight of tubers of any one variety.

(b) Environment.

It has already been pointed out that the dry matter, total nitrogen, and starch contents of the potato tuber vary with environment. In order to see how far environment influenced the tyrosinase p.cresol reaction of mature tubers, experiments were arranged whereby potato plants were grown in soils of various types and of varying degrees of acidity, also in widely separated localities and altitudes. Mature tubers were obtained from the plants grown at the seven centres in Midlothian described in the section dealing with "Maturity". The soil types varied from light garden soils at A and D to clay loams at B and C. The manurial treatments were essentially the same in all cases amounting to 20-25 tons per acre of farmyard manure and 8-10 cwts. of artificial fertilisers in the

usual proportions adopted for potatoes in this area. The plots at B received additional treatment to produce a wider range in acidity and will be considered separately. Localities B and F were over 500' above sea level, the remainder being between 100' and 200' O.D. A few tubers together with a sample of soil were taken from beneath each plant after ripening. The samples were taken to the laboratory where the tubers were treated with standard p.cresol reagent and a constant obtained for the velocity of red colour formation. The soils were air dried, after which a pH determination was made for each sample by means of the quinhydrone electrode (3,25). In this way it was found possible to compare the reactivity of tyrosinase to the p.cresol reagent with altitude, soil type and soil acidity. A summary of the results is given in Table 11.

At locality B, five plots each 1/200th acre, were laid down and specially treated to produce a wide range in soil acidity. Two of the plots were dressed with flowers of sulphur which is biochemically oxidised in the soil to sulphuric acid (26). A third plot was untreated while the remaining two were made alkaline by treating them with calcium hydroxide. The treatments were made at the end of March, a month before planting the potatoes. The varieties Duke of York and Great Scot were grown and typical plants were marked for investigation in each plot. By this

Table 11.

Relation between Tyrosinase - p.cresol Reaction of Mature Potato Tubers with Environment.

Locality	Altitude O.D.	Soil Type	Soil Acidity pH units	Duke of York		Epicure		Great Scot		Golden Wonder		Majestic		Ally	
				k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
A	200'	Garden	6.9	1.4	12.4	1.5	12.1	0.98	8.1	1.4	14.4	1.3	12.2	1.4	12.0
B	600'	Clay Loam	5.5	1.5	12.8	–	–	0.98	8.5	–	–	–	–	–	–
C	200'	Clay Loam	4.9	1.5	12.2	1.5	12.3	1.0	8.3	1.5	14.1	1.3	12.0	1.5	12.4
D	100'	Garden	5.4	1.5	13.3	1.5	12.4	0.99	7.8	1.5	14.5	1.3	12.5	1.4	12.3
E	100'	Medium Loam	6.4	–	–	1.4	11.8	–	–	–	–	–	–	1.4	12.6
F	500'	Clay Loam	5.3	1.5	12.9	–	–	–	–	–	–	1.4	12.5	–	–
G	100'	Sandy Loam	7.3	–	–	1.4	12.1	1.00	8.6	–	–	–	–	–	–

The figures given above for k and r_{10} represent the average of four values obtained from duplicate tubers from each of two plants.

The pH values represent the average acidity of the soils during September.

Table 12.

Relation between Tyrosinase p.Cresol Reaction of Mature Tubers and Soil Acidity at the centre (B).

Plot	Treatment	Soil Acidity pH units	Duke of York		Great Scot	
			k	r_{10}	k	r_{10}
A	10 tons $Ca(OH)_2$ per acre	8.1	1.5	12.8	0.96	8.3
B	$3\frac{1}{2}$ tons $Ca(OH)_2$ per acre	7.5	1.5	13.1	0.96	8.2
C	Untreated	5.5	1.5	12.5	0.98	8.5
D	800 lbs. sulphur per acre	5.1	1.5	12.6	0.97	8.3
E	1200 lbs. sulphur per acre	5.0	1.5	12.9	0.96	8.5

The values for the soil acidity varied throughout the growing season, and the pH values given above are averages of the September readings.

means it was possible to investigate the tyrosinase p.cresol reaction from tubers grown over a wide range of soil acidity. The results are given in Table 12.

An examination of the data of tables 11 and 12 indicated that, for potato plants grown within a comparatively small area, the tyrosinase activity of the tubers is independent of altitude, soil type and soil acidity.

In order to find out how far locality influenced the reaction, samples of mature tubers of a number of varieties were obtained in the Autumn of 1928 from the following counties. Aberdeenshire, Ayrshire, Banffshire, Dumfriesshire, Fifeshire, Midlothian and Perthshire.

The material was supplied by:-
Messrs.Alexander and Brown, Perth; Mr A.W.McAlister, Dumfries; Messrs. Dobbie and Co.Ltd.,Edinburgh; Messrs. McGill and Smith,Ltd.,Ayr; Department of Agriculture for Scotland,Corstorphine; Aberdeen and North of Scotland College of Agriculture,Aberdeen; and Edinburgh and East of Scotland College of Agriculture, Edinburgh.

The results obtained for a few varieties grown at the above localities are given in Table 13. A complete record is included in the section dealing with "Variety" (appendix, p.68.).

It would appear that the influence of locality upon the tyrosinase reaction is negligible except in a few cases which will be considered in detail in the discussion of the results.

Table 13.

Relation between Tyrosinase p.Cresol Reaction of Mature Tubers and Locality.

Variety	Aberdeenshire		Banffshire		Perthshire		Midlothian		Dumfriesshire		Ayrshire.	
	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
Arran Comrade	1·0	9·8	1·1	10·1	1·0	10·0	1·1	10·5	1·1	10·6	1·2	11·2
Duke of York	1·4	12·5	1·4	12·4	1·5	13·0	1·5	12·7	1·5	12·9	1·5	13·0
Golden Wonder	1·5	14·6	1·5	14·3	1·5	14·5	1·5	14·8	–	–	1·6	16·0
President	1·4	16·5	–	–	1·5	16·5	1·5	16·8	–	–	–	–
Rhoderick Dhu	1·0	9·3	1·0	9·0	1·0	9·3	1·2	10·9	1·1	9·8	–	–
Tinwald Perfection	1·3	12·5	1·3	12·6	1·3	12·4	1·3	13·3	1·3	12·8	1·4	13·0

Each figure given above represents the average value for six tubers.

(c) Variety. From a consideration of the results already noted in the sections dealing with Maturity and Environment, it is obvious that the tyrosinase activity of the mature potato tuber depends to a large extent upon variety and is independent of environmental influences. In order to determine the extent of such varietal effects, samples of tubers of a number of the commoner potato varieties were obtained in Autumn 1928 from seven localities in Scotland, extending from Banffshire to Ayrshire. The counties in which the crops were grown, together with the growers concerned, have been mentioned previously in section (b). On arrival at the laboratory the samples of each variety were examined closely, and twenty five to thirty sound and typical tubers selected (injured or diseased tubers were rejected). From these at least six were picked at random and treated with p cresol reagent as already described. A few examples have been given in Table 13 and the complete statement of results is given in Appendix p. 68.

In order to simplify the completed table of results it was decided to classify the varieties according to the colour of the skin of the tubers. Five groups were formed (a) White varieties (b) Blue varieties (c) white varieties with pink eyes, (d) pink varieties, and (e) white varieties with purple eyes.

Except in two cases, the influence of variety on the tyrosinase activity of the tubers was constant

for all the varieties examined. The two exceptions, namely Arran Chief and Up to Date will be considered in more detail in the "Discussion of Results".

(d) Disease etc. It was recognised that before the tyrosinase p.cresol reaction was used to identify potato varieties it must be possible with any one variety to obtain reproducible results with every tuber which is capable of sprouting and thus producing a plant. It has been shown that concordant results were obtained when sound and healthy tubers were selected for the test. Commercial stocks however often contain a considerable percentage of tubers which have been (i) attacked by diseases such as "Common Scab" (Actinomyces scabies (Thaxt) Gusson), Corky Scab (Spongospora subterranea, Lagerh) Blight (Phytophthora infestans (Mont) DeBy), Blackleg (Bacillus atrosepticus, van Hall) Sprain (Bacterium ruberaciens).

(ii) injured during or after harvesting.

(iii) greened through exposure to light.

all of which are capable of producing plants.

A number of injured, diseased and greened tubers were collected and treated with the cresol reagent. A summary of the results obtained is given in Table 14(a) and shows that the course of the reaction is influenced, sometimes to a large extent, by disease, injury and greening.

This suggested that the cresol reagent could be used to detect diseased tubers of a given

Table 14 (a)

Relation between Tyrosinase p.Cresol Reaction and Disease, etc.

Variety	Healthy Tuber		Affected Tuber		Nature of disease etc. of tuber.	Remarks on Colour Formation.
	k	r_{10}	k	r_{10}		
Great Scot	0·98	8·5	1·1	10·5	Severe common scab	Very dense at cortex.
Great Scot	0·98	8·5	1·3	12·6	Severe corky scab	"
Duke of York	1·5	12·7	1·7	14·5	Corky scab	Dense near affected part.
Majestic	1·3	12·4	1·6	16·0	Blight	Irregular
Duke of York	1·5	13·0	1·6	14·2	Blight	"
Epicure	1·5	12·4	1·6	13·2	Blackleg	Dense at heel-end.
Epicure	1·5	12·1	1·7	14·5	Severe sprain	Dense round affected areas.
Kerr's Pink	0·54	5·5	0·95	7·8	Sprain	"
Arran Chief	1·0	9·4	1·4	12·5	Injured by grape	Dense near injury.
Majestic	1·3	12·5	1·7	16·5	Cut across by digger	Very regular.
Arran Chief	1·0	9·4	0·96	8·5	Greened	Little colour produced near cortex.
Great Scot	0·98	8·5	0·84	7·6	Greened on one side	Little colour produced on greened side.

Note. Most of the diseased tubers assumed a "dried" appearance a few minutes after treatment.

Table 14 (b).

Relation between Tyrosinase p.Cresol Reaction and Virus Infection.

Variety	Nature of Infection.	k	r_{10}	Remarks on Colour Formation.
Ally	Healthy	1.4	12.5	Regular
	Leaf Roll	1.5	13.8	Irregular.
	Mild Mosaic	1.4	12.8	"
	Crinkle	2.1	17.3	Dark round cortex.
Arran Comrade	Healthy	1.1	10.9	Regular
	Mild Mosaic	1.1	11.2	"
	Severe Mosaic	1.3	13.5	Darker round cortex.
	Severe Leaf Roll	1.2	12.8	"
President	Healthy	1.5	16.5	Regular
	Leaf Roll	1.5	17.2	"
	Mosaic	1.5	17.4	"
	Intervernal Mosaic	2.2	22.0	Irregular.

Note. The velocity of colour formation in infected tubers gradually diminished after about 9 minutes when the surface of the section assumed a "dried" appearance. The values given above were obtained from readings taken up to nine minutes after treatment of the section.

variety. Such a method of detection would be of exceptional value if successfully applied to tubers infected with virus diseases such as "Leaf Roll" and "Mosaic", which unlike the diseases mentioned above in (i) are unrecognisible in the tuber stage. Accordingly plants suffering from the effects of leaf roll, mosaic and crinkle were marked during the growing season. In the autumn the diseased plants along with a neighbouring healthy haulm were harvested and the tyrosinase activity of the crops determined. The results are given in Table 14 (b). The data show that the milder forms of virus infection have very little effect on the course of the reaction. The more severe forms such as severe mosaic and crinkle do influence the tyrosinase activity of the infected tubers.

(e) Season. In the autumn of 1929 samples of tubers ofthe varieties examined the previous year were again obtained from Aberdeenshire, Perthshire, Midlothian, Dumfriesshire and Ayrshire. The season 1929 was characterised by exceptionally favourable conditions for the growth of the potato plant, and in consequence a large crop of healthy, well formed tubers resulted. The yield was well above average. In comparison, the season of 1928 was much less favourable, the crop being much lighter than in the following year. If, as is possible, a seasonal variation in the tyrosinase

activity occurs, then it should be apparent from an examination of tubers grown in the seasons 1928 and 1929. the conditions of which form a direct contrast. The results for the course of the reaction, which were obtained from tubers of the same varieties, grown at the same source in both seasons, are given in Table 15. A complete statement of results for the 1928 and 1929 crops is given in the Appendix, pages 68 and 69 respectively.

A consideration of the data indicates that little or no seasonal variation exists.

(f) Storage. After the potato crop has been harvested, the method of storage which is adopted depends upon such factors as variety, accomodation and weather conditions. Thus early varieties, which are in some cases difficult to keep in a healthy condition throughout the winter, are frequently stored in shallow boxes or trays in a cool, dry, storehouse. The commoner commercial varieties, nowever, are usually stored in a pit in the open, or within a storehouse if adequate accomodation exists. Since it is possible that the varying conditions of storage may bring about different chemical reactions or alter the course of the same reaction, it was considered advisable to investigate the tyrosinase p.cresol reaction of tubers stored in a number of different ways. With this end in view a number of tubers of each of the six varieties, Duke of York, Epicure, Great Scot, Golden Wonder,

Table 15.

Relation between Tyrosinase p.Cresol Reaction and Season.

Variety	Source	Season 1928		Season 1929	
		k	r_{10}	k	r_{10}
Ally	Midlothian	1.5	12.5	1.5	12.4
Arran Comrade	Perthshire	1.0	10.0	1.1	10.9
Edzell Blue	Ayrshire	1.9	16.8	1.9	17.3
King Edward	Dumfriesshire	0.44	4.6	0.49	5.0
Tinwald Perfection	Aberdeenshire	1.3	12.5	1.4	12.9

Readings given above represent the average values of six tubers.

Majestic, and Ally were placed in a pit and covered with straw and soil in the manner adopted on most farms. A second series was placed in bags in a cool, well ventilated storehouse, while a third set was stored in a refrigerator, the temperature of which was maintained at 2°C. The three sets of samples were stored in the autumn of 1929. In the spring of 1930 tubers of each variety were removed from their various storage quarters and after standing in the laboratory for one day were treated with cresol reagent. The results, which are given in Table 16 show that the tyrosinase activity of "pitted" tubers closely approximates that of tubers kept in the storehouse, and further that the values obtained are in good agreement with those of the same stocks before storage. It was found that storage at 2°C. retarded the course of the reaction. Tubers examined about one hour after removal from the refrigerator were found to have little reactivity towards the reagent. A similar but much smaller inhibition was observed in tubers taken directly from the pit. In both cases, however, the reaction assumed it normal course after the tubers had been allowed to stand in a warm atmosphere (15°C.) for about 24 hours.

Table 16.

Relation between Tyrosinase p.Cresol Reaction and Storage Conditions.

Variety	Examined before Storage, Oct.1929		Examined after Storage in Pit. April, 1930.		Examined after Storage in Storehouse April 1930.		Examined after Storage in Refrigerator April, 1930.	
	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
Duke of York	1.5	12.2	1.5	12.0	1.5	12.6	1.4	11.0
Epicure	1.5	12.3	1.5	12.1	1.5	12.5	1.3	11.2
Great Scot	1.0	8.3	0.98	8.5	0.97	8.0	0.96	7.9
Golden Wonder	1.5	14.1	1.4	14.0	1.5	14.8	1.3	13.2
Majestic	1.3	12.0	1.2	11.8	1.3	12.8	1.1	10.8
Ally	1.5	12.4	1.4	12.0	1.5	12.6	1.3	10.6

The figures given above represent the average values of six tubers.

3. Discussion of Results.

From a consideration of the graphs for the rate of the tyrosinase p.cresol reaction it is obvious that no one law is applicable throughout. The greater part is logarithmic, but the initial and final stages are linear. In this respect the reaction resembles other enzymic decompositions (18 section X). It is convenient to observe the first linear portion which occurs within a period of 15-20 minutes after application of the reagent. The form of this part of the curve, where the substrate is in excess, suggests that the reaction velocity is proportional to the concentration of enzyme. In the case of the potato tuber such a proportionality cannot be assumed in view of the fact that other substances are present which may interfere with the course of the reaction. For instance it has been shown that peroxidase retards (38) while o-dihydric phenols accelerate (36) the action of tyrosinase. Peroxidase and a homologue of catechol (35) have both been identified in the potato tuber. Hence tubers which are very reactive towards p.cresol may owe their reactivity to the presence of a catechol. On the other hand it is possible that some varieties contain a relatively large percentage of tyrosinase the presence of which is concealed by inhibitors.

The behaviour of the varieties President and Golden Wonder suggests the possible existence

of varietal differences in Tyrosine content. When tubers of either variety are cut and allowed to stand very little blackening takes place (Table 3). Since the blackening is caused by the action of tyrosinase or tyrosine this result would indicate the paucity of either enzyme or amino acid. When treated with cresol however both varieties are very reactive indicating a high concentration of enzyme, or the presence of an accelerator. In a similar manner it can be deduced that Majestic is rich both in enzyme and amino acid; Eclipse is rich in amino acid but contains a low concentration of enzyme or an active inhibitor.

The effect of maturity on the tyrosinase cresol reaction can be divided into three stages,

(1) A rapid fall in reaction velocity.

(2) A minimum activity, the position of which varies with environment and variety, but which occurs after the plant has reached maximum growth.

(3) A slight increase in activity which becomes constant after the plant ceases to grow.

The following explanation may be put forward to explain the changes of activity. It has been noted (49) that normal tuber formation usually commences somewhere about the period of maximum growth of the haulm. Assuming this to be correct for the potatoes under observation the very small and reactive tubers would be formed when the plant is at or has just passed the point of maximum growth. After this

period the rate of growth falls off until complete maturity is reached. The observed diminution in tyrosinase activity of the tubers occurs about this period in the life of the plant and may quite well accompany the decreasing growth rate. It has been suggested frequently that oxidising enzymes are closely connected with respiratory functions, further it is known that respiration is very great at the period of maximum growth of the plant. It is possible therefore that the greater oxidase activity of young tubers is in some way related to the vigorous respiration of the fully grown plant. As the plant approaches maturity the respiration activity falls off as does the enzyme activity of the tuber. Finally when the plant is completely mature the respiration and also the enzyme activity are at their minimum values. Up to this stage in its development the tuber has been dependent on the growing plant for sustenance. When maturity is reached however the tuber severs its connection with the haulm and commences a separate existence, and after a short time assumes its own characteristic properties. It is about this period that the rise in tyrosinase activity to a constant value is noticed. If it is the case that the minimum tyrosinase activity coincides with complete maturity of the plant, then an observation of the enzyme would provide an accurate method of estimation of the property "Maturity" which is at present uncertain on account of the arbitrary methods of its determination.

With mature tubers it was noted that environment and season had little or no influence on the oxidase activity. This is to be expected if the enzyme action is connected with respiration which should be constant for normal individuals of one type. Disease and injury increase the tyrosinase activity. It is well known that injured tissue shows greater respiratory activity than normal tissue, so that again there would appear to be a close connection between tyrosinase activity and respiration. "Greening" reduces the enzyme activity, but it also increases the solanine content of the tuber. The presence of the increased quantity of alkaloid may be responsible for the inhibition of the enzyme of "greened" tubers. With regard to storage effects a decrease in tyrosinase activity was observed after long storage in a pit or at low temperature. After a short time the activity returned to approximately the normal value. This may also be explained on the basis of respiration. The respiration activity of plant tissue which has been stored at low temperatures is very great for a short period after being reintroduced to a normal atmosphere. A rapid increase in respiration can bring about the rapid accumulation of decomposition products, such as carbon dioxide, which would tend to retard other processes. (19 p. 74). It may be pointed out here that the temporary decrease in enzyme activity on removal from storage was accompanied by a temporary increase in acidity (page 56).

The results obtained show that variety determines to a large extent the tyrosinase activity of a potato tuber. Hence the methods described for the estimation of enzyme activity can be made to provide evidence for the detection of variety. With this end in view the results given in the appendix pages 68,69 have been rearranged and are presented in Table 17.

For each variety column "b" gives the average of the average values from all sources. The figures in column "a" were obtained by deducting three times the standard error from the lowest of the average values from all sources, while those in column "c" were obtained by adding three times the standard error to the highest average value of all sources. Thus any tuber having a value lower than "a" or higher than "c" for any variety is significantly different from that variety.

It is possible to separate two varieties when the value of "c" for one is equal to or less than the value of "a" for the other. For example Great Scot having a value of "c" equal to 9.8 units can be distinguished with certainty from Ally which has a value for "a" of 11.0 units. Great Scot, however, cannot with confidence be separated from Arran Comrade which has a value for "a" of 8.8 units, and cannot be distinguished from Royal Kidney for which "a" is 8.0 units.

Table 17.

The Differentiation of Potato Tubers.

White Varieties			
Variety	a	b	c
Dunotter Castle	1.0	2.5	4.0
Eclipse	4.0	5.5	7.0
May Queen	4.5	6.0	7.5
Great Scot	7.0	8.5	9.8
Up-to-Date	5.8	8.5	12.3
Royal Kidney	8.0	9.5	11.0
Witchhill	8.0	9.5	11.0
Rhoderick Dhu	8.0	9.8	12.0
Arran Chief	8.0	10.2	13.0
Arran Comrade	8.8	10.5	12.2
Lochar	9.0	10.5	12.0
Epicure	10.5	12.0	13.5
Ally	11.0	12.5	14.0
Majestic	11.0	12.5	14.0
Duke of York	11.2	12.8	14.3
Tinwald Perfection	11.5	12.8	14.3
Abundance	11.5	13.0	14.5
Immune Ashleaf	12.6	14.3	16.0
Champion	13.0	14.5	16.0
Golden Wonder	13.0	14.5	16.0
Crusader	15.0	16.5	18.0
President	15.5	16.5	18.0

Pink Varieties			
Variety	a	b	c
Kerr's Pink	4.5	6	7.5
Reading Russet	7.5	9	10.5
Rogue like Great Scot	8.5	10	11.5
Orange Anther Substitute	9.5	11	12.5
Epicure	10.5	12	13.5
Gregor Cups	13.5	15	16.5
Snarpe's Pink Seedling	17.5	19	20.5
Raeburn's Gregor Cups	19.5	21	22.5

Blue Varieties			
Variety	a	b	c
Arran Victory.	7.5	9.0	10.5
Edzell Blue	15.5	17.0	18.5

White Varieties with Pink Eyes			
Variety	a	b	c
King Edward	3.5	4.5	6.0
K of K	5.5	7.0	8.5
Katie Glover	16.0	17.5	19.0

White Varieties with Purple Eyes.			
Variety	a	b	c
Catriona	11.0	12.5	14.0
Di-Vernon	15.0	17.5	19.5

It is interesting to note that Kerr's Pink can be distinguished without any difficulty from any of the other pink varieties with which it is so often confused. Similarly Arran Victory can be separated from Edzell Blue and King Edward from Katie Glover. So far such distinctions can only be made by observation of the growing plants.

Two varieties, British Queen and King George, were examined but could not be given values for k and r_{10} on account of the irregular colouring of the tuber section. Both varieties were very much more reactive in the cortex than in the medulla, a point which proves of value in their detection. Three other varieties Arran Chief, Up-to-Date and Di-Vernon did not give concordant results. All three varieties are extremely susceptible to leaf diseases such as mosaic and leaf roll while the first two are also susceptible to wart disease infection. With regard to Di-Vernon it appears that a number of types are at present included under the name and it is not known whether the variation has been caused by disease or whether more than one variety exists.

4. SUMMARY.

A number of preliminary experiments have been described in which chemical reagents were employed in an attempt to discriminate varieties.

One of the reagents (p cresol) has been selected and a satisfactory laboratory technique developed. This enabled the rate of decomposition of the cresol by tyrosinase in the potato tissue to be followed by observing with a tintometer the formation of an orange coloured intermediate product.

The course of the tyrosinase p.cresol reaction has been shown to be monomolecular with a short linear portion at the commencement. From an observation of this period of the reaction constants for the enzyme activity were obtained, and a temperature coefficient deduced.

The enzyme activity was shown to depend upon stage of maturity of tuber, variety of tuber, and disease infection but to be independent of environment, season, and storage conditions.

The probable significance of the results has been discussed and a table of varieties, grouped according to their enzyme activities, drawn up.

The Hydrogen Ion Concentration of the Potato Tuber.

In recent years the hydrogen ion concentration of plant sap has been made the subject of numerous investigations, and various methods have been devised for the accurate determination of this important value (9). An electrometric method embodying the hydrogen electrode in conjunction with a standard calomel half-cell has been extensively used in plant work (11,17). This combination, however, has several disadvantages a few of which may be mentioned. In the first place the time taken for the sap to come into equilibrium with the hydroge n is about 15 minutes; and during this period enzymic decomposition may have proceeded a considerable distance. It is possible that acidity changes may accompany such reactions. In the second place some writers (9 p.444) are of the opinion that the rapid flow of hydrogen through biological systems contained in an open vessel, alters the composition of the fluid by causing loss of gaseous products such as carbon dioxide. The loss of some of the components necessarily upsets the equilibrium of the system. Another objection is the rapid "poisoning" by the plant products, of the platinum black with which the electrode is coated. To avoid such difficulties indicators have been employed to determine the hydrogen ion concentration of plant sap. (31). Determinations of the pH of potato sap have been obtained in this way

(10,33) but the methods are difficult to apply on account of enzyme action which renders the fluid opaque and coloured. Within the last few years there has been a rapid development of the quinhydrone half cell (3) which has been applied successfully in a wide field of acidity determinations. The electrode possesses many advantages, the most important of which is the rapid attainment of equilibrium enabling measurements to be made in a very short time. One possible disadvantage is suggested. Chodat (8) demonstrated that plant oxidases, such as are present in the potato, attack hydroquinone. If this reaction takes place when quinhydrone is added to potato sap, the ratio hydroquinone/quinone, the constancy of which is necessary for accurate use of the electrode, will be disturbed. Equilibrium is attained so quickly, however, that unless the decomposition is exceedingly rapid, it should be possible to make an observation before the ratio of reductant to oxidant is appreciably disturbed. Accordingly it was decided to carry out acidity determinations with potato sap, using the quinhydrone electrode and to compare the results with those obtained with the standard hydrogen system.

The examination was divided into sections as follows:-

1. Laboratory Methods. (a) Extraction of Sap from Tubers.
   (b) Hydrogen Electrode Measurements.
   (c) Quinhydrone Electrode Measurements.
   (d) Construction and Use of a Micro-Electrode.

2. Factors Influencing the pH of Potato Tubers.
   (a) Variation of pH throughout the tuber.
   (b) Maturity
   (c) Environment
   (d) Variety
   (e) Disease
   (f) Storage

3. Discussion of Results.

4. Summary.

1.(a) Extraction of Sap from Tubers. The following method was adopted for the extraction of the sap. The tubers under examination were washed in cold water, dried lightly with a cloth, and pulped by means of an electrically driven pulper. A fine mash was obtained which was used in the preliminary experiments. This practice,however, was found to be unsatisfactory owing to the presence of small pieces of tissue in the mash. These adhered to, and frequently blocked the electrodes, bringing about a large increase in the resistance of the cell. Furthermore a considerable time was required for the hydrogen-calomel cell to reach equilibrium when used in conjunction with the

mash. In order to surmount such difficulties the mash was filtered through fine linen, and the filtrate employed in the pH determinations. The filtered sap was found to be satisfactory and was accordingly used in preference to the mash.

Decomposition of the pulped tubers was rapid, so that the sap usually possessed a red brown colour, which darkened on standing.

(b) Hydrogen Electrode Measurements.

In making the measurements with the hydrogen electrode the following combination was used:

Pt H | Plant Sap | KCl (sat.) HgCl | Hg

The hydrogen electrode was of the Hildebrand type with a platinum foil coated with platinum black. The black was deposited by electrolysis from a solution of platinic chloride after which the electrode was placed in a dilute solution of sulphuric acid to remove traces of platinic chloride by electrolysis and to displace absorbed chlorine with hydrogen. Before being used the electrode was thoroughly washed with boiled distilled water. After each measurement it was washed, cleaned with filter paper and reblackened. A "saturated" calomel half cell was used to complete the circuit. In order to minimise diffusion of potassium chloride, the tip of the electrode was drawn out to a fine capillary and then bent into the form.

The vessel was connected to a reservoir of saturated potassium chloride - calomel and by means of a side tube and stopcock could be flushed out after each determination. The tip of the electrode was placed in direct contact with the plant sap, the saturated potassium chloride solution in the vessel taking the place of the usual connecting solution.

The procedure was as follows:- About 5 c.c. of the plant sap were placed in a small narrow beaker and a current of hydrogen passed through by means of a delivery tube. Losses due to frothing were prevented by placing a cone of filter paper in the mouth of the beaker. After approximately 5 minutes, the hydrogen electrode was substituted for the delivery tube and the flow of hydrogen gas continued until equilibrium was reached. The circuit was completed by dipping the calomel electrode into the sap, and the e.m.f. of the combination observed by the use of a potentiometer system. The pH of the sap was calculated from the voltage of the cell, temperature corrections being applied where necessary. The instrument was calibrated frequently with standard buffer solutions.

(c) Quinhydrone Electrode Measurements.

The combination consisted of saturated calomel and quinhydrone elements.

Pt	Quinhydrone, Plant Sap (sat.)	KCl sat.	HgCl	Hg

The quinhydrone half cell consisted of a stout platinum electrode which was placed in the potato sap saturated with quinhydrone. Before using, the electrode was cleaned in hot chromic acid, washed with distilled water and heated to redness in an alcohol flame. The saturated calomel electrode has already been described in the previous section. Estimates were carried out as follows. A small quantity of quinhydrone (the correct amount was determined experimentally) and 10 c.c. of potato sap were placed in a test tube and shaken vigorously. The contents of the tube were immediately transferred to a small beaker and the platinum and calomel electrodes lowered into the solution. The e.m.f. of the cell was observed on the potentiometer circuit and the pH calculated. The whole operation was carried out in less than a minute, but even in such a short time the decompsition of the phenol had commenced. After each determination the platinum electrode was washed and heated to redness in an alcohol flame. The apparatus was checked before and after use with standard phosphate and phthalate buffer solutions. A preliminary experiment was carried out to determine the amount of quinhydrone which must be added to 10 c.c. potato sap in order to produce a constant potential. Varying amounts of quinhydrone were added to test tubes each containing 10 c.c. of sap and the acidity determined as before. The values given below were obtained from

the sap of two different varieties and indicate that at least 0.12 gms. quinhydrone must be added to 10 c.c. sap to obtain reproducible results.

Wt. of Quinhydrone in gms.	0.01	0.025	0.05	0.075	0.10	0.125	0.15
pH value of (1)	4.79	5.38	5.61	5.71	5.72	5.72	5.72
potato sap (2)	-	5.33	5.49	5.57	5.65	5.66	5.66

In order to compare the quinhydrone-calomel and hydrogen-calomel combinations the sap obtained from a number os samples of potatoes was determined by both methods. A few of the results are given in Table 1, and in the Appendix p.71.

Table 1.

Comparison of Quinhydrone-Calomel and Hydrogen-Calomel combinations in Determination of pH of Potato Sap.

Sample	pH Value	
	Quinhydrone-Calomel	Hydrogen-Calomel
1.	5.66	5.72
2.	5.62	5.71
3.	5.62	5.67
4.	5.73	5.64
5.	5.61	5.67

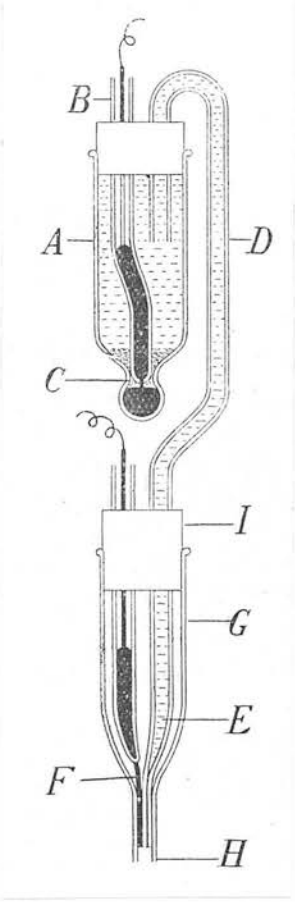
The results obtained by the two methods are in fairly good agreement. In the majority of cases

(83%) a slightly higher pH value was observed with the hydrogen electrode . This may be due to a loss of CO_2 from the sap during the longer period required to make a measurement with the hydrogen electrode system, or to some error in the application of the quinhydrone cell. In any case the use of the quinhydrone element enabled reproducible results to be obtained with great ease and rapidity, and was used in all subsequent determinations.

(d) Two difficulties were encountered in the course of a study of the hydrogen-ion concentration of potato tubers by the methods already described. In the first place , the time required to prepare a pulp was sufficient to allow enzyme action to proceed to a considerable distance, and the disintegrated tissue could not with confidence be regarded as possessing the same degree of acidity as the original tissue. In the second place, there is no evidence to show that the pH value is uniform throughout the potato tuber. The apparatus described in this section was designed to meet those difficulties.

Description of Apparatus. The accompanying diagram is drawn to scale. The vessel A is a small "saturated" calomel half-cell, the rounded end of the tube B, which carried the connecting wire, fitting the constriction at C fairly closely so that the mercury is not disturbed by tilting. D is a saturated potassium chloride-agar bridge ending in a fine capillary at E. This

capillary is sufficiently long to permit of the periodic removal of small pieces from the end, so as to expose a fresh surface of potassium chloride-agar. The platinum wire F, heaten out at the end, lies close to and protects the point E. It is kept in position by the tube G, which is drawn out at H so that the internal diameter is less than 1 mm. The ends of the wire F and the capillary E reach a point about 2 mm. from the end of the tube G. The whole apparatus is only 14 cm. long and weighs about 40 g., so that it can be held like a pencil.



The procedure adopted was to place a few crystals of quinhydrone on the tissue at the point under investigation and then insert the point H. The plant juice, together with some quinhydrone, rose in the capillary to meet E and F and the potential difference was determined by means of a potentiometer circuit, the accuracy of which was about 0.5 millivolt.

To clean the apparatus for another determination, the tube G was slipped off the rubber stopper I and a jet of water directed where necessary. In addition, the stopper was partially split so that the tube carrying F could be removed easily to permit of

the wire being flamed without endangering the capillary E.

Statement of results. A comparison of values obtained for four buffer solutions by this capillary electrode and by means of the quinnydrone-calomel system-previously mentioned is presented in Table 2.

Table 2.

pH Values of certain buffer solutions.

Capillary Electrode	Ordinary Electrode
3.98	3.99
4.92	4.92
6.07	6.06
7.09	7.07

Groups of five tubers were examined by this method and the average pH value of the tissue compared with that of the pulp prepared from the same groups of tubers. A few results are given in Table 3, which illustrate typical differences obtained.

Table 3.

Average pH of tissue obtained by capillary electrode compared with the pH of pulp.

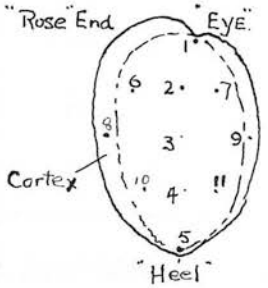
Tissue	Pulp
5.68	5.76
5.70	5.76
5.68	5.74
5.67	5.71

It will be observed that the hydrogen-ion concentration of the pulp is always lower than that of the tissue; this point is discussed later.

2. Factors Influencing the Acidity of the Potato Tuber.

(a) Variation of acidity throughout the tuber.

There is much evidence to show that the acidity of the sap may vary throughout the members of a plant. Thus Gustafson (16) was able to trace a gradient in hydrogen-ion concentration in a number of plants, which suggests the possibility of the existence of a similar variation in the potato tuber. As no observations seem to have been made on this subject it was decided to make use of the micro-electrode to investigate the acidity at different points within a number of tubers. The tuber under examination was cut along a plane parallel to the axis joining the "rose" and "heel" ends, and the acidity determined at eleven points on the section. The positions were chosen so that four were inside the cortex, and the remaining seven at different positions throughout the medulla. The approximate positions are shown in the diagram. The observations, a few of which are given in Table 4, indicate that at this period of the year (November) tubers do show a variation in pH value, being most acid in that part of the cortex in the vicinity of the eyes, and least acid at the heel. Generally the average of the eleven readings for a tuber closely approximates to the value obtained for the centre of



the tuber; accordingly in subsequent determinations the pH value for the centre of tuber was observed and taken as being representative of the whole.

Table 4. Variation of pH value over a section of potato tuber.

Position on Tuber	Tuber 1	Tuber 2	Tuber 3	Tuber 4	Tuber 5
1	5.46	5.79	5.67	5.67	5.55
2	.48	.81	.69	.69	.60
3	.53	.84	.69	.70	.62
4	.53	.86	.70	.70	.62
5	.57	.88	.72	.74	.62
6	.53	.84	.70	.69	.59
7	.53	.84	.70	.69	.57
8	.48	.81	.70	.69	.55
9	.45	.81	.69	.67	.55
10	.53	.88	.70	.74	.59
11	.48	.88	.70	.74	.60
Average	5.51	5.84	5.70	5.70	5.59

(b) Maturity. In order to, investigate the effect of stage of maturity on the pH value of potato tubers, a number of healthy and typical plants of the varieties Duke of York and Great Scot were selected early in the growing season and marked for intensive examination. Samples of tubers were taken from each plant at intervals throughout the summer and the acidity

determined by means of the micro-electrode; readings being made in the centre of each tuber. A few of the results are given in Table 5.

Table 5.

Relation between pH value and Tuber Maturity.

Variety	Plant	Date of Sampling.					
		20.7.29	11.8.29	3.9.29	9.9.29	18.9.29	11.10.29
Duke of York	1	6.41	6.36	6.07		5.89	
	2	6.35	6.33	6.01		5.86	
	3	6.40	6.31	5.98		5.84	
	4	6.30	6.24	6.00		5.82	
	5	6.28	6.24	6.11		5.84	
Great Scot	1	6.06	6.03	5.86	5.80		5.72
	2	5.90	6.00	5.88	5.84		5.70
	3	6.04	6.03	5.83	5.79		5.70
	4	6.16	5.96	5.84	5.77		5.68
	5	5.90	6.00	5.90	5.79		5.72

It would appear from the observations that the acidity of the tuber increases with stage of maturity, slowly at first and then more rapidly as the plant approaches complete maturity.

(c) Environment. The effect of environment upon the hydrogen ion concentration of potato tissue was determined for mature tubers grown at the seven sources in Midlothian which have been mentioned already under the Tyrosinase p.Cresol Reaction in section (b.) Environment. The mature tubers were harvested towards the end of September and in October and were taken to the laboratory where the acidity was determined immediately, that is within a few hours after removal from the soil. The values were obtained with the micro electrode, a reading being taken in the centre of each tuber. Table 6 gives a summary of the results. There is no definite relation between acidity of the tuber sap and environment. In order to examine more fully the effect of soil reaction upon the pH of potato sap, the crops grown on specially treated plots at locality B were employed. The determinations extended over two seasons, a different method of treatment being employed each year.

In 1928 an area in which the soil was very acid was subdivided into a number of small plots, each 1/250 acre, which were then treated with different quantities of different calcium compounds. The resulting soil reaction of the plots ranged from a pH value of 4.2 units (untreated) to 6.8 units. The variety King George was planted throughout the area and in October a number of plants in each plot were harvested. At the same time representative samples

Table 6.

Relation between Reaction of Sap of Mature Potato Tubers with Environment.

Locality	Altitude	Soil Type	Soil Acidity pH Units.	Duke of York	Epicure	Great Scot	Golden Wonder	Majestic	Ally.
A	200'	Garden	6.9	5.50 ± 0.03	5.75 ± 0.03	6.04 ± 0.06	5.74 ± 0.02	5.54 ± 0.04	5.70 ± 0.03
B	600'	Clay Loam	5.5	5.84 ± 0.05	-	5.70 ± 0.05	-	-	-
C	200'	Clay Loam	4.9	5.83 ± 0.04	5.80 ± 0.05	6.03 ± 0.08	5.80 ± 0.05	5.68 ± 0.05	5.75 ± 0.02
D	100'	Garden	5.4	5.76 ± 0.06	5.74 ± 0.04	5.87 ± 0.07	5.73 ± 0.03	5.64 ± 0.02	5.79 ± 0.03
E	100'	Medium Loam	6.4	-	-	-	-	-	5.79 ± 0.05
F	500'	Clay Loam	5.3	5.81 ± 0.02	-	-	-	5.61 ± 0.03	-
G	100'	Sandy Loam	7.3	-	-	5.71 ± 0.05	-	-	-
Maximum variation of individual Values from all Sources.				5.70 - 5.89	5.70 - 5.85	5.65 - 6.11	5.70 — 5.85	5.50 — 5.63	5.67 — 5.84

The pH values for the soils represent the average acidity during September.

The pH values for the tuber sap represent the average acidity of four tubers.

of the soil round each plant was collected. A few tubers from each plot were washed and pulped as already described (page 41) after which the pH of the sap was determined by means of the hydrogen-calomel, and quinhydrone-calomel systems (see pages 42 and 43) The results are given in the appendix p. 71.

In 1929 another series of five plots was laid down (see p. 25.) on a soil which had a pH value of about 5.5 units. Two plots were made acid and two alkaline by treatment with sulphur and calcium hydroxide respectively. The varieties Duke of York and Great Scot were planted in each plot. In October a number of tubers of those varieties were lifted from each plot and the acidity of the tissue determined with the micro electrode. In both series of experiments the pH of the soil was determined with the quinhydrone-calomel system. The results are given in Table 7.

An examination of the data given in Tables 6 and 7 indicates that for potato plants grown within a comparatively small area, the pH of the sap of the tubers is independent of altitude and soil conditions.

Table 7.

Relation between the Acidity of Tuber Tissue and the Acidity of the Soil.

Plot	Treatment	Soil Acidity pH units	Acidity of Tuber Tissue.	
			Duke of York	Great Scot.
A	10 tons $Ca(OH)_2$ per acre	8.1	5.70	5.68
B	3½ tons $Ca(OH)_2$ per acre	7.5	5.68	5.69
C	Untreated	5.5	5.70	5.69
D	800 lbs Sulphur per acre	5.1	5.70	5.68
E	1200 lbs Sulphur per acre	5.0	5.71	5.68

The values for the soild acidity varied throughout the growing season and the pH values given above are averages of the September readings.

The values for the Acidity of the tuber tissue given above represent the averages of ten tubers.

(d) Variety.

From a consideration of the average values given in table 6 it would appear that varietal differences in acidity do exist. These however are small when compared with the variation which was shown by members of any one variety. The tubers of Majestic consistently yielded pH figures lower than those of the other five varieties, but even here the highest

pH value obtained for Majestic closely approximated to the lowest value for Great Scot. A few others varieties were examined and average pH values obtained as follows.

Arran Consul 5.64; Field Marsnal 5.74;
Kerr's Pink 5.75; King Edward 5.95.

The values however, were not constant for tubers of any one variety, a variation of as much as 0.30 pH unit being obtained in some cases. Since such large variations exist for one variety, it was felt that acidity determinations could not with any confidence be used to discriminate varieties.

(e) Disease. A number of tubers affected by various diseases were collected and their acidity compared with the acidity of normal tubers of the same variety. Examples of tubers suffering from "Corky Scab", "Blight", "Blackleg", "Sprain", and "Wart Disease" are given in Table 8 (a). Tubers affected by those diseases had a very acid region in the tissue round about the diseased part. The other parts of the tuber appeared to be almost normal except in very severe cases, when a slight increase in acidity was observed throughout the tuber. It is interesting to note that blight in the early stages of development slightly decreases the acidity of the tuber, but after the tubers have been stored for some time, the region around the affected part becomes more acid than the healthy tissue.

Table 8 (a).

Relation between pH Value of Tuber Sap and Disease.

Variety	Nature of Infection	Normal Tuber.	Affected Tuber.	
			Healthy Part	Diseased Part
Great Scot	Corky Scab	5.70	5.65	4.35
Duke of York	Common Scab	5.75	5,70	4.58
Epicure	Sprain	5.73	-	5.60
Epicure	Black Leg	5.73	5.68	5.65
Duke of York	Blight (fresh)	5.75	5.75	5.83
Duke of York	Blight (old)	5.75	5.70	5.38
Duke of York	Wart Disease	5.75	5.65	5.02

Table 8 (b).

Relation between Acidity of Tuber Sap and Virus Infection.

Variet y	Nature of Infection	pH value of tuber sap.
Ally	Healthy	5.80
	Mosaic	5.70
	Crinkle	5.60
	Leaf Roll	5.85
Arran Comrade	Healthy	5.64
	Mosaic	5.47
	Leaf Roll	5.70

The above figures represent averages of four tubers.

With regard to the effect of "Virus Diseases" on the pH value of tuber tissue a few observations are noted in Table 8 (b). In the case of "Leaf Roll" a slight increase in pH value was noted. With the severe forms of Mosaic such as "Crinkle" an increase in acidity resulted; the milder forms of mosaic had very little effect on the acidity of t he tissue.

(f) Storage. In the course of a preliminary experiment it was noted that storage conditions were responsible for large acidity changes in the potato tuber. Thus there was a decrease in acidity of tubers stored for a few weeks in a warm room. In order to obtain precise information on this subject a number of tubers of each of the six varieties, Duke of York, Epicure, Great Scot, Golden Wonder, Majestic and Ally was placed in a pit in the open. A second set was placed in bags in a cool, well ventilated storehouse and a third set was stored in a refrigerator at 2°C. After a few months had elapsed samples were taken from each series and the acidity of the tubers determined. The results (Table 9) showed that the tubers stored in the storehouse became less acid. Those in the pit and the refrigerator were acid when examined immediately, but on standing for some time (usually about 24 hours) the pH figure increased to a value usually slightly higher than the original.

Table 9.

Relation between Acidity of Tuber Tissue and Storage Conditions.

Variety	pH Value in Oct.1929.	Examined after storage in Store-house	Examined after Storage in Pit		Examined after Storage in Refrigerator	
			Immediately	After 24 hours.	Immediately	After 24 hours.
Duke of York	5.84	5.90	5.43	5.97	5.65	5.95
Epicure	5.80	5.85	5.65	5.87	5.52	5.78
Great Scot	5.70	5.82	5.40	5.93	5.42	6.01
Golden Wonder	5.80	5.88	5.42	5.80	5.12	5.78
Majestic	5.68	5.75	5.50	5.95	5.05	5.67
Ally	5.75	5.83	5.52	5.75	5.55	5.80

The figures given above represent the averages of six tubers.

The analyses for the stored material was carried out in March 1930.

3. Discussion of Results.

Attention has already been drawn to the fact that tuber tissue examined directly with the micro electrode is consistently more acid than the pulp obtained from the same tubers, which may be accounted for as follows. The loss of carbon dioxide present in the cell sap during the process of pulping probably results in a decreased hydrogen ion concentration. Ingold (24) has estimated that the presence of carbon dioxide in concentrations such as occur in the intercellular spaces of the tuber may alter the pH value 0.3 - 0.4 units. Furthermore the oxidase activity in the disintegrated tissue is very great and according to Raper (40) brings about the conversion of tyrosine to indole derivatives which are intermediate products in the formation of melanin. It is not known exactly how far those transition compounds would influence the hydrogen ion concentration, but formation of the final insoluble product, melanin, would doubtless reduce the acidity.

The variation throughout the tuber is to be expected, if consideration is given to the different functions performed by the various parts. Thus the region in the vicinity of the eyes is intimately associated with the growing parts which are usually more acid than fully developed members. The acidity of growing tubers increases with maturity: this is the reverse of the acidity changes in the growing

shoot, which is most acid in its early stages of development. Appleman and Miller (1) observed a somewhat similar contrariety, the younger tubers containing protein which gave place to non protein and amine-nitrogen in the mature state.

The acidity of the potato tuber does not appear to be affected by environmental factors such as soil acidity. This is remarkable when it is considered that the soils under examination ranged from pH 4 to pH 8. Similar results for other plants have been obtained by Hoagland and Davis (23) and Reed and Haas (42). After long storage in a pit or at low temperature, tubers showed an increased acidity which might be due to accumulation of acid products during storage or to decomposition products produced by the greater respiration activity which takes place when plant tissue is removed from storage conditions. After remaining in a normal atmosphere for 24 hours the pH value increased to a value slightly higher than that obtained for the same variety before storage.

There would appear to be a slight variation in hydrogen ion concentration of tuber sap with variety but this is not large and when compared with the variation within the members of any one variety is not significant. Hence the estimation of pH value cannot be used to discriminate varieties.

4. Summary.

The quinhydrone electrode has been used to determine the acidity of tuber sap and has been checked with the standard hydrogen electrode system. In addition a micro-quinhydrone calomel cell has been devised to observe the hydrogen ion concentration at a point on plant tissue.

It has been shown that the acidity varies with position in the tuber, maturity and variety of tuber, disease infection, and storage conditions. The pH value of tuber sap was found to be independent of environmental conditions such as soil acidity.

The variation with variety however was not significantly greater than that of the tubers of any one variety, so that the determination of hydrogen ion concentration of tuber sap could not be employed to differentiate varieties.

Pectic Substances.

From a consideration of Table 17 it will be observed that a number of the white varieties of tubers cannot be distinguished by means of the p.cresol reagent. For example Great Scot and Arran Chief two of the most popular varieties in Scotland cannot be distinguished by this test. Similarly it is impossible to separate British Queen from King George or Tinwald Perfection from Up-to-Date (some samples of Up-to-Date and Tinwald Perfection could be separated by p.cresol but on account of the variable results obtained with the former variety such a separation was not always certain). The above pairs of varieties have very similar tubers and moreover are often confused in the field on account of the similarity of their haulms. In order to attempt a separation of such varieties a number of pectin stains were applied to potato sections. Mangin (18) discovered that phenosafranin and bismarck brown in neutral or slightly acid solution stained pectic acid but not cellulose. For example safranin coloured the pectic substances orange red and the protoplasm and corky material red. These stains however also coloured nitrogenous bodies but treatment with alcohol or acids decolorised the pectic substances but not the nitrogenous substances. Nigrosin and indulin stained the nitrogenous material but not the pectic

substances. Four solutions were made up as follows:- ½% phenosafranin and bismarck brown in water, I% Nigrosin and indulin in water.

A few drops of each reagent were added to sections of potato tuber and allowed to stand for 15 minutes after which excess reagent was washed off. It was found that while a deep colouration was obtained with phenosafranin and bismarck brown only a feeble stain was produced with nigrosin and indulin. On treatment with alcohol most of the staining of phenosafranin and bismarck brown was removed. These observations indicated the presence of pectic substances in the potato tuber. Ten tubers of each of the varieties, Great Scot, Arran Chief, British Queen, King George, Tinwald Perfection and Up-to-Date from different sources were treated with phenosafranin and allowed to stand ten minutes after which the excess reagent was washed off with water. The intensity of the orange colour produced was estimated with the tintometer, and the following average results obtained.

Source	Great Scot		Arran Chief		British Queen		King George		Tinwald P.		Up-to-Date.	
	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow	Red	Yellow.
Aberdeen-shire	11.5	6.4	14.1	5.8	14.2	5.8	14.5	6.0	16.3	6.0	13.7	6.0
Banffshire	11.3	6.2	14.0	5.2	-	-	-	-	16.1	6.0	12.8	6.0
Perthshire	11.5	6.0	-	-	-	-	-	-	16.6	6.0	15.2	6.0
Midlothian	11.8	6.3	14.3	5.0	14.6	5.6	14.3	5.8	16.8	6.0	16.2	6.0

The values for British Queen and King George are practically identical. Those for Tinwald Perfection are constant but again there is a variation with Up-to-Date. This test however is useful in the separation of Great Scot from Arran Chief, every tuber of Arran Chief which was examined being darker in colour than those of Great Scot.

Phenolic and Amino Substances.

The possible existence of varietal differences in phenolic and amino substances in the potato tuber has been discussed on page 32 . In order to get more definite information, a potato tuber was pulped under alcohol and the mash filtered. The filtrate was concentrated and made alkaline, and then treated with a small amount of diazotised sulphanilic acid when a red colour was produced.

The test was repeated with equal weights of potato tuber obtained from the variet ies Golden Wonder and Ally which were placed in Nessler tubes and covered with a definite amount of alcoholic NaOH. After 10 minutes a few drops of a concentrated solution of diazo-benzene sulphonic acid were added. The red colour produced was darker with Ally than Golden Wonder. On repeating the experiment with the same varieties from another source the same result was obtained.

This preliminary experiment suggested that

further investigation would yield valuable information, applicable to the discrimination of potato varieties. It was not found possible to make a more detailed examination of the reaction.

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APPENDIX.

Reaction Velocity of Tyrosinase p.Cresol Reaction.

Colour Intensities produced on potato sections with solutions of p.cresol containing half the equivalent quantity of sodium hydroxide.

(a) Variety - Rhoderick Dhu. Reagent - 1.0% p.cresol in ½ equivalent NaOH.

Time Interval t	Intensity of Red Colour r_t	Intensity of Yellow Colour Observed	Intensity of Yellow Colour Corrected y_t	k_r	k_y
0 min.	-	2.5	-	-	-
5 "	2.0	3.0	0.5	0.030	0.032
10	3.8	3.3	0.8	.030	.025
15	5.4	3.6	1.1	.031	.025
20	7.0	4.2	1.7	.034	.033
25	8.4	4.2	1.7	.035	.027
30	9.6	4.6	2.1	.036	.030
35	10.4	5.0	2.5	.036	.036
40	11.1	5.2	2.7	.037	.037
45	11.6	5.4	2.9	.037	.039
50	12.2	5.7	3.2	.037	.049
55	12.5	5.8	3.3	.037	.052
60	12.9	5.8	3.3	.037	.047
65	13.2	6.0	y_∞ 3.5	.037	-
70	13.4	6.0	3.5	.037	-
75	13.5	6.0	3.5	.036	-
80	13.7	6.0	3.5	.036	-
85	13.9	6.0	3.5	.036	-
150	r_∞ 14.5	6.0	3.5	-	-

(b) Variety - Duke of York. Solution - 0.5% p.Cresol in $\frac{1}{2}$ equivalent NaOH.

Time Interval t	Intensity of Red Colour r_t	Intensity of Yellow Colour Observed	Corrected y_t	k_r	k_y
0 min.	-	2.7	-	-	-
5	2.3	4.2	1.5	0.056	0.134
10	3.8	4.2	1.5	.052	.067
15	4.9	4.6	1.9	.049	.063
20	5.8	4.6	1.9	.048	.047
25	6.6	4.6	1.9	.048	.038
30	7.2	5.0	2.3	.048	.046
35	7.6	5.0	2.3	.047	.040
40	8.0	5.4	2.7	.048	.050
45	8.4	5.4	2.7	.050	.046
50	8.6	5.4	2.7	.049	.041
55	8.8	5.4	2.7	.050	.037
60	8.9	5.4	2.7	.049	.034
65	9.0	5.8	y_∞ 3.1	.049	-
70	9.1	5.8	3.1	.049	-
75	9.2	5.8	3.1	.051	-
120	r_∞ 9.4	5.8	3.1	-	-
150	9.4	5.8	3.1	-	-

Temperature Coefficient of the Tyrosinase p.Cresol Reaction.

(a) Velocity constants (k) for the formation of red colour, produced at different temperatures with a 2% solution of p.cresol containing half the equivalent quantity of sodium hydroxide.

Variety	Temperature	$k = \frac{dr}{dt}$
Tinwald Perfection	8°C.	0.57
" "	13°	0.96
" "	18°	1.30
" "	23°	1.79
" "	28°	1.98
Edzell Blue	13°	1.23
" "	23°	2.24

(b) Temperature Coefficients of the reaction at different temperature ranges.

Variety	Temperature Range.	Temperature Coefficient.
Tinwald Perfection	$8^{\circ} - 18^{\circ}$	2.28
" "	$13^{\circ} - 23^{\circ}$	1.86
" "	$18^{\circ} - 28^{\circ}$	1.52
Edzell Blue	$13^{\circ} - 23^{\circ}$	1.82

Relation between Tyrosinase p.cresol Reaction and Tuber Maturity.

Locality	Date of Sampling.	Duke of York		Epicure		Great Scot		Golden Wonder		Majestic		Ally	
		k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
A	27. 6.29	2·5	20·0	1·7	14·8	–	–	–	–	2·0	21·4	2·1	18·0
	6. 7.29	–	–	–	–	2·2	19·0	1·9	21·0	–	–	–	–
	28. 7.29	1·7	14·6	1·3	10·2	1·8	16·0	1·8	17·4	2·0	19·1	1·6	13·7
	4. 9.29	1·5	12·0	1·4	11·7	0·96	8·7	1·5	12·6	1·0	9·5	1·1	9·0
	7.10.29	1·4	12·4	1·5	12·1	0·98	8·1	1·4	14·4	1·3	12·2	1·4	12·6
B	20.7.29	2·2	19·2			2·7	24·5						
	11.8.29	1·3	12·0			1·8	16·1						
	3.9.29	1·4	12·3			0·94	8·9						
	9.929	–	–			0·98	9·0						
	18.9.29	1·5	12·8			0·98	8·5						
C	28. 6.29	–	–	1·9	16·5	2·8	24·0	–	–	–	–	–	–
	12. 7.29	2·3	21·0	–	–	–	–	–	–	2·2	22·0	2·0	17·6
	19. 7.29	–	–	–	–	–	–	2·0	21·8	–	–	–	–
	4.8.29	1·8	15·5	1·5	10·4	1·7	14·9	1·9	18·0	1·9	18·7	1·8	14·0
	5. 9.29	1·5	12·5	1·5	12·3	0·93	8·5	1·4	12·4	1·7	15·3	1·6	14·5
	4.10.29	1·5	12·2	1·5	12·3	1·0	8·3	1·5	14·1	1·3	12·0	1·5	12·4

Relation between Tyrosinase p.cresol Reaction and Tuber Maturity.

Locality	Date of Sampling.	Duke of York		Epicure		Great Scot		Golden Wonder		Majestic		Ally	
		k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
A	27. 6.29	2·5	20·0	1·7	14·8	–	–	–	–	2·0	21·4	2·1	18·0
	6. 7.29	–	–	–	–	2·2	19·0	1·9	21·0	–	–	–	–
	28. 7.29	1·7	14·6	1·3	10·2	1·8	16·0	1·8	17·4	2·0	19·1	1·6	13·7
	4. 9.29	1·5	12·0	1·4	11·7	0·96	8·7	1·5	12·6	1·0	9·5	1·1	9·0
	7.10.29	1·4	12·4	1·5	12·1	0·98	8·1	1·4	14·4	1·3	12·2	1·4	12·6
B	20.7.29	2·2	19·2			2·7	24·5						
	11.8.29	1·3	12·0			1·8	16·1						
	3.9.29	1·4	12·3			0·94	8·9						
	9.929	–	–			0·98	9·0						
	18.9.29	1·5	12·8			0·98	8·5						
C	28. 6.29	–	–	1·9	16·5	2·8	24·0	–	–	–	–	–	–
	12. 7.29	2·3	21·0	–	–	–	–	–	–	2·2	22·0	2·0	17·6
	19. 7.29	–	–	–	–	–	–	2·0	21·8	–	–	–	–
	4.8.29	1·8	15·5	1·5	10·4	1·7	14·9	1·9	18·0	1·9	18·7	1·8	14·0
	5. 9.29	1·5	12·5	1·5	12·3	0·93	8·5	1·4	12·4	1·7	15·3	1·6	14·5
	4.10.29	1·5	12·2	1·5	12·3	1·0	8·3	1·5	14·1	1·3	12·0	1·5	13·4
D	24. 6.29	2·2	18·6	1·8	15·1	–	–	–	–	2·1	22·6	1·7	15·1
	4. 7.29	–	–	–	–	2·3	21·5	2·3	25·8	–	–	–	–
	25. 7.29	1·8	15·0	1·2	9·5	2·0	17·0	2·0	19·0	1·9	18·4	1·6	11·2
	6. 9.29	1·4	12·2	1·5	12·0	0·99	8·6	1·5	13·5	1·3	11·8	1·4	12·4
	4.10.29	1·5	13·3	1·5	12·4	0·99	7·8	1·5	14·5	1·3	12·5	1·4	12·3
E	4. 7.29			1·9	16·6							1·9	17·0
	2. 8.29			1·8	11·1							1·7	14·4
	22. 8.29			1·4	11·8							–	–
	4.10.29			–	–							1·4	13·6
F	12. 7.29	2·5	21·8							2·0	21·8		
	1. 8.29	1·9	15·1							1·6	18·2		
	20. 9.29	1·5	12·9							1·4	12·5		
G	25. 6.29			1·7	15.2	–	–						
	4. 7.29			–	–	2·6	4·0						
	13. 7.29			1·1	10·2	–	–						
	28. 8.29			1·4	12·1	–	–						
	5.10.29			–	–	1·0	8·6						

The figures given above represent the average of four values obtained from duplicate tubers from each of two plants.

Relation between Tyrosinase p.Cresol Reaction of Mature Tubers grown at different Localities and Variety. (1928 crop).

(a) White Tuber Varieties.

Variety.	Aberdeenshire		Banffshire		Perthshire		Midlothian				Dumfries-shire		Ayrshire	
	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
Abundance	1·4	13·2	1·3	12·5	1·4	13·1	1·4	13·3	1·5	13·0	1·3	12·7	–	–
Ally	–	–	–	–	–	–	1·5	12·5	1·5	13·0	–	–	–	–
Arran Chief	1·2	12·2	1·4	12·1	1·2	11·4	1·0	9·2	–	–	0·98	9·5	1·0	9·7
Arran Comrade	1·0	9·8	1·1	10·1	1·0	10·0	1·1	10·5	–	–	1·1	10·6	1·2	11·2
Champion	–	–	1·4	14·1	–	–	1·4	14·4	–	–	–	–	–	–
Crusader	1·7	16·3	–	–	1·7	16·2	1·8	16·8	–	–	1·7	16·4	–	–
Duke of York	1·4	12·5	1·4	12·4	1·5	13·0	–	–	1·5	12·7	1·5	12·9	1·5	13·0
Dunnotter Castle	–	–	–	–	–	–	0·35	2·4	–	–	–	–	–	–
Eclipse	–	–	–	–	–	–	0·70	5·3	–	–	–	–	–	–
Golden Wonder	1·5	14·6*	1·5	14·3	1·5	14·5	1·5	14·6	1·5	14·8	–	–	1·6	16·0
Great Scot	0·99	7·8	1·0	8·2	0·93	7·9	0·98	8·2	1·0	8·4	0·99	8·4	0·98	8·4
Immune Ashleaf	1·4	14·0	1·4	14·2	–	–	1·3	13·6	–	–	–	–	1·5	15·4
Lochar	–	–	–	–	–	–	–	–	–	–	1·1	10·4	–	–
Majestic	–	–	–	–	–	–	1·4	13·4	–	–	–	–	–	–
May Queen	–	–	–	–	–	–	0·60	6·2	–	–	–	–	–	–
President	1·4	16·5	–	–	1·5	16·5	1·5	16·8	–	–	–	–	–	–
Rhoderick Dhu	1·0	9·3	1·0	9·0	1·0	9·3	1·2	10·9	–	–	1·1	9·8	–	–
Royal Kidney	0·98	9·0	–	–	–	–	–	–	–	–	0·98	9·4	–	–
Tinwald Perfection.	1·3	12·5	1·3	12·6	1·3	12·4	1·3	13·3	–	–	1·3	12·8	1·4	13·0
Up-to-Date	0·75	7·2	0·70	7·3	1·2	11·2	1·2	11·0	0·93	8·7+	0·75	6·9	0·68	6·8
Witchhill	–	–	–	–	–	–	1·1	9·5	–	–	–	–	–	–

* Langworthy
\+ Field Marshal

(b) Blue Tuber Varieties.

Arran Victory	1·1	9·4	1·1	8·7	1·1	8·8	1·1	8·9	1·1	9·0	1·0	9·0	–	–
Edzell Blue	1·8	16·8	1·8	16·9	1·8	16·9	1·9	17·1	1·8	16·9	1·9	16·6	1·9	16·8

(c) White Tuber Varieties with Pink Eye.

Katie Glover	1·8	17·2	–	–	–	–	1·9	17·9	–	–	1·9	17·7	–	–
K. of K.	–	–	–	–	–	–	0·70	7·4	0·60	6·6	–	–	–	–
King Edward	0·46	4·5	0·49	4·6	0·48	4·5	0·46	4·4	0·46	4·7	0·44	4·6	0·46	4·8

(d) Pink Tuber Varieties. (Midlothian only).

Gregor Cups	–	–	1·5	14·9
Kerr's Pink	0·69	6·3	0·54	5·5
Orange Anther Substitute	–	–	1·2	10·7
Raeburn's Gregor Cups	1·9	21·0	–	–
Reading Russet	–	–	1·1	8·8
Rogue like Great Scot	–	–	1·1	9·6
Sharpe's Pink Seedling	2·0	18·8	1·9	18·6

(e) White Tuber Varieties with Purple Eye.

Catriona	1·3	12·5	1·4	12·4
Di-Vernon	2·1	18·5	1·6	16·2

The figures given above were obtained from the average values of six tubers

Relation between Tyrosinase p.Cresol Reaction of Mature Tubers, grown at Different Localities, and Variety (1929 Crop).

(a) White Tuber Varieties.

Variety	Aberdeenshire		Perthshire		Midlothian				Dumfriesshire		Ayrshire.	
	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}	k	r_{10}
Abundance	1·4	12·9	1·4	12·7	1·5	13·5	1·4	13·3	1·4	12·5	–	–
Ally	–	–	–	–	1·5	12·4	1·4	12·0	–	–	–	–
Arran Chief	1·2	11·6	1·2	12·4	0·98	9·0	–	–	–	–	1·2	11·6
Arran Comrade	1·1	10·2	1·1	10·9	–	–	1·1	9·8	1·2	11·2	1·1	11·0
Crusader	1·7	16·5	1·6	15·8	1·7	17·0	–	–	–	–	–	–
Duke of York	–	–	–	–	1·5	12·2	1·4	12·4	–	–	–	–
Eclipse	0·62	5·5	0·67	5·0	–	–	0·72	5·9	0·70	6·1	0·65	5·8
Epicure	–	–	–	–	1·5	12·3	1·5	12·1	–	–	–	–
Golden Wonder	–	–	–	–	1·5	14·1	1·4	14·4	–	–	–	–
Great Scot	–	–	–	–	1·0	8·3	0·98	8·1	–	–	–	–
Immune Ashleaf	1·4	14·6	1·5	15·0	–	–	1·5	13·5	–	–	1·5	15·1
Majestic	–	–	–	–	1·3	12·0	1·3	12·2	–	–	–	–
President	1·5	16·6	1·5	16·9	1·4	17·1	1·4	16·5	–	–	–	–
Rhoderick Dhu	1·1	10·2	–	–	1·0	9·1	–	–	1·0	10·8	–	–
Royal Kidney	0·90	8·6	0·99	9·2	0·92	9·5	–	–	0·96	10·0	–	–
Tinwald Perfection	1·4	12·9	1·3	12·6	–	–	–	–	1·3	12·5	1·3	13·2
Up to Date	0·91	8·4	1·0	9·4	1·0	10·6	1·2	11·2	0·81	7·5	–	–

(b) Blue Tuber Varieties.

Arran Victory	1·0	18·9	1·1	9·0	1·0	8·5	1·1	9·5	1·1	9·2	–	–
Edzell Blue	1·8	16·6	1·9	16·8	–	–	1·9	17·0	1·8	17·0	1·9	17·3

(c) White Tuber Varieties with Pink Eye.

Katie Glover	1·9	17·2	1·7	17·5	1·9	17·5	–	–	–	–	–	–
King Edward	0·48	4·6	0·57	4·8	–	–	0·45	4·2	0·49	5·0	0·42	4·6

(d) Pink Tuber Varieties.

Variety	Midlothian.			
	k	r_{10}	k	r_{10}
Gregor Cups.	1·5	15·3	1·4	14·4
Kerr's Pink	0·68	6·8	0·59	5·8
Roguelike Great Scot	1·1	9·8	1·1	10·5
Raeburn's Gregor Cups.	2·0	21·5	1·9	20·2

(e) White Tuber Varieties with Purple Eyes

Catriona	1·4	12·8	1·3	11·8
Di-Vernon	2·0	17·8	1·6	14·6

Estimation of Standard Error of the Tyrosinase p.Cresol Reaction.

The standard errors for the intensity of red colour after 10 minutes (r_{10}) were obtained for a few varieties by the method detailed by Fisher (14).

Let $\bar{X}$ = the arithmetic mean of each set of n individual values.

and X = any one value.

then $(X - \bar{X})$ = the deviation from the mean and

$\Sigma(X - \bar{X})^2$ = the sum of the squares of the deviations

$$\sigma^2 = \frac{\Sigma(X - \bar{X})^2}{n - 1} = \text{the variance}$$

$$\sigma = \sqrt{\frac{\Sigma(X - \bar{X})^2}{n - 1}} = \text{the standard deviation}$$

$$\frac{\sigma}{\sqrt{n}} = \text{the standard error of the mean.}$$

In each case six tubers of a variety were investigated. From the readings determined from each tuber a graph was drawn and values of r_{10} obtained. The standard error of the mean and a percentage error were calculated for each set of six tubers, and a few examples are given below.

Deviations from the mean which exceed more than three times the standard error are considered significant.

Variety	r_{10}		
	Mean	$\sigma/\sqrt{n}$	% error
Abundance	12.7	0.29	2.27
Arran Comrade	10.7	0.35	3.28
Crusader	15.3	0.32	2.11
Arran Victory	9.0	0.21	2.33

Determination, by hydrogen-calomel and quinhydrone-calomel systems, of the acidity of cell sap of tubers grown in soils of different reaction.

Number of Plot	pH value of soil.	pH Value of Potato Sap.	
		Hydrogen-Calomel System	Quinhydrone- Calomel System
28	4.18	5.70	5.67
19	4.29	5.64	5.70
34	4.33	5.65	5.72
17	4.56	5.63	5.67
16	4.65	5.67	5.71
8	4.68	5.60	5.67
26	4.74	5.69	5.69
14	4.75	5.59	5.62
9	4.79	5.67	5.70
27	4.79	5.66	5.71
18	4.96	5.61	5.64
23	5.05	5.60	5.64
24	5.08	5.64	5.57
1	5.08	5.66	5.72
29	5.08	5.60	5.65
5	5.35	5.61	5.67
4	5.41	5.73	5.64
22	5.51	5.58	5.66
3	5.75	5.62	5.67
7	5.80	5.64	5.72
30	5.83	5.64	5.67
32	6.28	5.52	5.55
12	6.48	5.63	5.66
6	6.72	5.68	5.63

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