STUDIES ON THE GROWTH PROMOTING SUBSTANCE OF THE EXCISED WHEAT ROOTS II. CHANGES IN THE BENEFICIAL EFFECTS OF PEPTONE BY THE FRACTIONATION

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II. CHANGES IN THE BENEFICIAL EFFECTS OF PEPTONE BY THE FRACTIONATION

By

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It was reported in the previous paper (3) that neopeptone was markedly effective on the growth of the excised wheat roots. The effects of peptone were observed not only in the main axis length, the production and the development of the laterals and the root weight, but also metabolically in nutrient uptake, respiratory rate and nitrogen contents of the excised roots. Moreover, it was emphasized from a histological observation that any substance contained in peptone may be referred to as a hormonal or stimulant substance for the cell division in the roots.

Yeast extract and peptone have been well employed as a mixture of substances, including nitrogen compounds, various vitamins and the other organic compounds beneficial for the culture of plant tissues, and it is clear that not all of the constituents of these substances are essential for the satisfactory medium. Robbins (4) and Almestrand (1) found that peptone was favorable for the growth of the excised roots, and Robbins and Schmidt (5) demonstrated with the excised tomato roots that peptone at a sufficiently high concentration was a substitute for yeast extract for the growth and that the beneficial effects of these compounds were mostly due to vitamin B_1 contained in them. White (6) also found that yeast extract was favorable for the growth of the excised tomato roots. In order to identify the beneficial constituents in yeast extracts, he separated from yeast the material essential for the growth by various preparations and cleared (8) that vitamin B_1 played the greatest parts for the growth of excised tomato roots.

We place great emphasis on the investigation whether our results on the wheat roots are like those of White and Robbins on tomato or corn roots in the nature of beneficial constituents of peptone for the excised wheat roots. In this paper, peptone was fractionated in some constituents by physical or

chemical preparations, and the effects of their constituents on the growth were observed with the culture of the excised wheat roots.

Matarials and Methods

As our experimental materials the roots of wheat, variety *Norin No. 55* were used. The culture methods of the excised roots were largely due to the results stated in the previous report (3). The root tips excised from wheat seedling were aseptically cultured in the nutrient solution containing 2 per cent of glucose with inorganic ions and B vitamins.

Peptone constituents fractionated or decomposed were respectively employed with the amount equivalent to peptone 300 mg as dry weight or about 30 mg as nitrogenous matter per liter, and all of the results in this paper were obtained by the culture period of two weeks. The other experimental methods in this study are described with the contents of results.

Results and Conclusion

Experiment 1. Relation between peptone and B vitamins. In the basal medium, thiamin 0.1, pyridoxine 0.5 and nicotinic acid 0.5 mg per liter are contained as micro-organic elements. These B vitamins have been well known as growth factor for the excied roots of many dicotyledonous plants, but are nonbeneficial for those monocotyledonous. As shown previously by authors (2), pyridoxine was slightly beneficial for the growth of the main axis tips. It is readily supposed, however, that peptone effects are not originated from B vitamins contained in it, because the roots cease growth during shorter period in spite of the presence of vitamins, and the addition of peptone to the medium with vitamins prolongs the growth period.

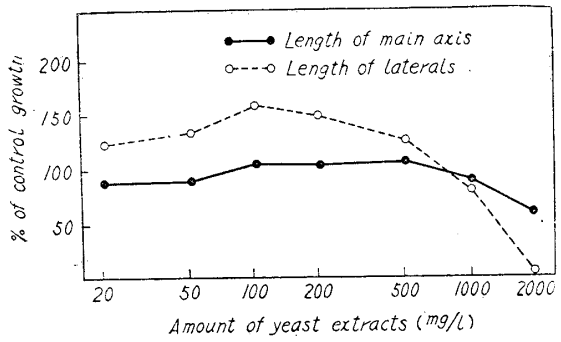

Fig. 1. Effects of yeast extracts on the growth of the excised roots.

Fig. 1 shows the results of the excised roots grown in the presence of different amounts of yeast extracts substituted for the B vitamins, *i.e.* thiamin, pyridoxine and nicotinic acid.

Five grams of dried beer yeast was boild in 100 cc of water for an hour, cooled and then centrifuged to take off the insoluble materials. Different amounts of supernatant liquid were added to the basal medium whitout vitamins.

The growth of the laterals were most excellent in the culture medium containing an amount of the extract equivarent to 100 mg of dry yeast per

liter, but the effects on the growth of the main axis tips were negligible. Amounts over 500 mg per liter of yeast extract seem to be excess for satisfactory growth on account of the abnormal swelling of the root tips.

In order to compare the effects of peptone with those of yeast, influences of the addition of yeast extract and peptone to the medium with or without vitamins were investigated by the culture of the excised roots. The results are shown in Fig. 2. Peptone was employed at a concentration of

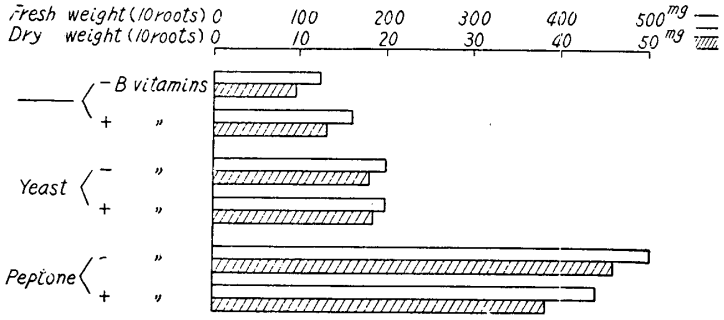

Fig. 2. Influence of peptone and yeast extract in the presence or the absence of B vitamins.
Peptone: 300 mg per liter. Yeast extract: Equivalent to the amount of 100 mg dry yeast per liter. B vitamins: thiamin 0.1, pyridoxine 0.5, nicotinic acid 0.5 mg per liter.

300 mg per liter, and yeast extract at a concentration equivalent to 100 mg of dry yeast per liter. The growth of the main axis tips in the presence of three vitamins were slightly superior to those in the absence of them and their beneficial effects on the growth of the main axis tips were found somewhat for two weeks. The effects of B vitamins appear to be compensated with the addition of yeast because of little difference in the growth between media with and without vitamins. The beneficial effects of yeast on the growth, however, were markedly inferior to those of peptone, although being excellent somewhat as compared to the effects of vitamins only. A few roots in the peptone solution without vitamins often ceased the growth of the main axis tips by the way of the culture process, and abnormally produced and developed the laterals. This may be a reason why the amounts of the root weights in the absence of vitamins are higher rather than in the presence of them.

Since vitamins, namely thiamin, pyridoxine and nicotinic acid cannot be a substitute for peptone for the growth of excised wheat roots, beneficial constituents of peptone may be different from these B vitamins. This does not agree with the results on tomato roots by Robbins *et al.* (5) and White (8), who found methods to substitute vitamin B_1 for peptone or yeast.

Experiment 2. Changes in the peptone effects by dialysis. Peptone was fractionated in two parts diffusible and nondiffusible with dialysis through a

cellophane membrane, and the effects of each constituent on the growth of the excised roots were observed.

Two grams of peptone was dissolved in 20 cc of deionized water and the solution was placed within a cellophane membrane, which was immersed in 200 cc of deionized water. The four parallel series were similarly prepared and each external water was renewed every hour during one, two, four or eight consecutive periods, those solutions being respectively mixed at the end of the preparation. Only one series was made by immersing the membrane in tap water during 24 hr.

Change in the diffusible amount of peptone with time was checked with decrement of nitrogen content in the residue whithin the membrane. As shown in Fig. 3, the amount of the dialyzed material increased gradually with time, coming up to about 80 per cent of the whole amount originally present within the membrane after 24 hr.

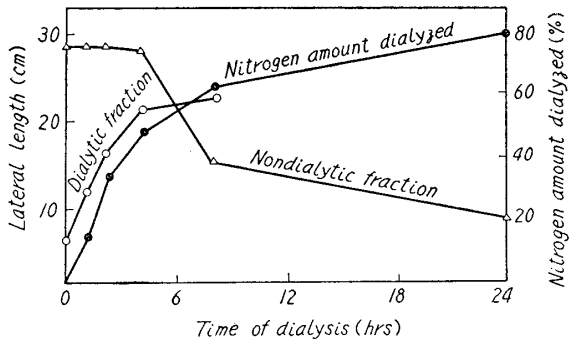

Fig. 3. Changes in the peptone effects by dialysis. The growth of the excised roots were represented with total length of laterals per root, in which peptone effects were most typically observed.

The growth promoting properties of the two fractions were shown by the data presented in Fig. 3, expressing the root growth by the lateral length in which the effects of peptone were most marked. In proportion to amount of the dialytic material, the activity of peptone for the growth moved from the nondiffusible fraction to the diffusible with time, being not found at all in the residue within the membrane after 24 hr.

It is thus clear that most of the effective materials of peptone pass through the membrane and that the residual nondiffusible materials are inert for the growth of the excised roots. From the results, the effective constituents of peptone may consist of lower molecular substances capable to pass through the cellophane membrane.

Experiment 3. Changes in the peptone effects with separation on the basis of alcohol or ether solubility. Peptone constituents, then, were separated on the basis of abcohol or ether solubility and the effects of each fraction on the growth of the excised roots were investigated.

The extraction of peptone with alcohol was performed as follows; 1 g of dried peptone was mixed with 50cc of absolute or 80 per cent alcohol, allowed to stand during 30 min at room temperature (about 15°C), mixed thoroughly and centrifuged. The supernatant solution, yellow in color, was evaporated

to dryness over a steam bath. The same preparation of alcohol extraction was repeated three times and the extraction with alcohol of peptone was thoroughly made. The extraction of peptone with ethyl ether was performed with soxhlet's apparatus for 16 hr.

The effects of the fractions on growth of the excised roots are shown in Table 1. Among all fractions, there were no great differences in length of the main axis, but notable in total length or number of laterals and root weight. The ether soluble fraction prepared in this way represented only about 0.8 per cent of the weight of dry peptone. The beneficial material for the growth was not in the soluble fraction, but remained largely in the insoluble fraction. The beneficial material in peptone, therefore, cannot be lipoidal in its nature, nor can it possibly be auxin soluble in ether.

Table 1. Changes in the peptone effects with alcohol and ether extraction.

Solvent	Fraction	Length of main axis (cm)	Length of laterals per root (cm)	No. of laterals per root	Fresh wt. per 10 roots (mg)	Dry wt. per 10 roots (mg)
Ether	soluble	14.6	2.1	5.2	31	7.4
	insoluble	16.0	37.6	20.0	503	38.0
Absolute alcohol	soluble	15.4	7.0	8.0	175	11.3
	insoluble	16.7	39.9	25.0	387	23.6
80% alcohol	soluble	16.4	9.6	10.8	220	16.3
	insoluble	16.5	40.7	20.1	412	25.8
Control		15.0	2.6	5.8	125	8.0
Peptone control		16.2	38.8	21.7	444	30.0

Solubilities of peptone in absolute and 80 per cent alcohol were respectively 13 per cent and 37 per cent under the given conditions. The effects of both soluble fractions on the growth also were not found at all like those of ether soluble fraction. In the fraction soluble in 80 per cent alcohol the growth was slightly excellent as compared with that in absolute alcohol, but inferior greatly to the insoluble fraction. On the other hand, all of the insoluble fractions in alcohol and ether were as effective as the peptone control. It is inferred from this experiment that the beneficial constituents of peptone have a character insoluble in ether and almost insoluble in absolute or 80 per cent alcohol.

Experiment 4. Separation of peptone constituents with paper chromatography. Under speculating amino acid group or peptide for the beneficial constituents, peptone was separated into some fractions with paperc hromatography and their effects on the growth were investigated.

The chromatograms were developed in the ascending manner with 80 per cent phenol solvent in room temperature. The filter papers used were *Toyo*

No. 50 (50×50 cm). When the solvent has travelled up as high as 30cm, the paper was dried and sprayed with ninhydrine. In another case, regions on unsprayed chromatograms corresponding to the colored regions were cut in three fractions as shown in Fig. 4, and were eluted respectively with water. In order to take off phenol, which may remain with the constituents on the paper, the pieces of filter were thoroughly dried in the desiccator for one week, and each liquid eluted was heated and dried over a steam bath before the addition to the medium.

Fig. 4 shows the influences on the growth of the peptone constituents separated with paper chromatography. With the addition of each fractionated

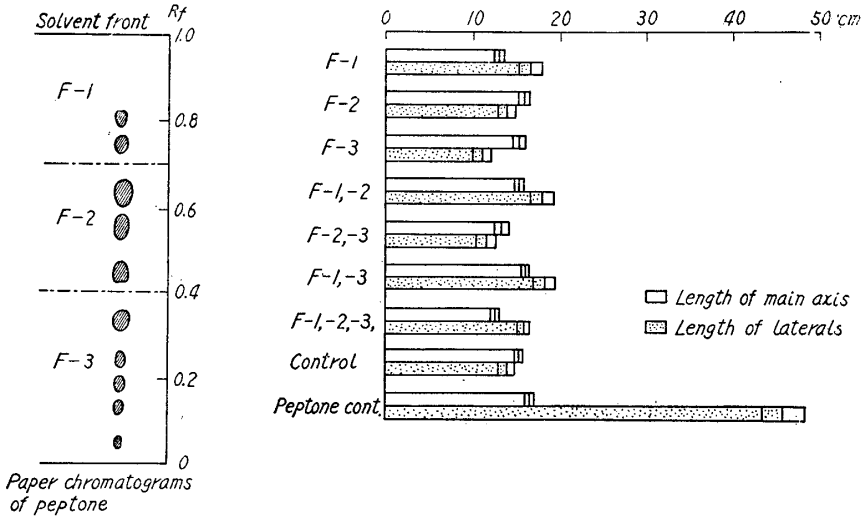

Fig. 4. Fractionation of peptone with paper chromatography and the effects of the fractionated constituents on the growth of the excised wheat roots. In each case the standard error is represented by vertical lines on either side of the mean value.

material or with the mixture from two or three fractions, beneficial effects could not be seen in the growth of the excised roots. Every constituents of peptone from the fractions were not beneficial, or apparently toxic somewhat from the observation in color of the roots. This may be due to some causes of the materials like peptide not to be dissolved or developed with the solvent, of the contamination of the developing solvent or some substance from the filter paper, and of the decomposition of beneficial substance with such factors as heating, lighting *etc.* during the preparation of paper chromatogram. Further experiments are nesessary to elucidate this matter.

Experiment 5. Stability of beneficial constituents of peptone with acid or alkali. Under the conditions usually used for the acid or the alkaline hydrolysis of pretein, peptone was digested. It was investigated, then, whether the digestion brings about a change in the beneficial effects on the growth.

Acid digestion : 2 g peptone was heated in 40 cc of 25 per cent H_2SO_4 at 100°C for 12 hr. Then the digested liquid was neutrized with barium hydroxide.

Alkaline digestion ; 5 g of peptone with 20 g of barium hydroxide was heated in 100 cc water at 102° or 105°C for 24 hr on the oil bath, and then the excess barium in the liquid was quantitatively taken off with sulfuric acid.

The peptone hydrolysate with acid or alkali was added in the limits of 7.5 to 120 mg as nitrogen per liter, their effects being tested with the culture of the excised roots. The results are shown in Table 2. In all of the different amounts of the acid hydrolysate of peptone, the lateral growth, the root

Table 2. Changes in the peptone effects with acid or alkaline digestion.

Growth / Treatment	Length of main axis (cm)	Length of laterals per root (cm)	No. of laterals per root	Fresh wt. per 10 roots (mg)	Dry wt. per 10 roots (mg)	Diameter* of root tip (mm)
Control	16.4	6.8	14.8	185	13.7	0.25
Peptone cont.	17.4	38.4	23.8	463	32.8	0.40
Acid hydrolysate						
7.5 mg N/*l*	18.2	12.1	12.5	223	18.1	0.25
15	19.4	16.1	14.5	290	23.4	0.25
30	18.7	19.0	15.8	295	23.2	0.26
60	15.4	22.4	14.8	255	19.4	0.27
120	15.1	12.9	17.3	240	15.7	0.27
Alkaline hydrolysate						
7.5 mg N/*l*	12.8	33.7	17.5	258	22.5	0.28
15	10.4	19.1	15.5	225	20.7	0.32
30	11.2	11.7	14.5	325	35.2	0.42
60	6.8	18.7	11.5	455	35.8	0.70
120	4.8	3.8	5.8	310	41.1	0.77

* Diameters of the main axis tips were mesured at 1mm from the apical part of the roots.

weights and the thickness of the root tips were less than those in the peptone control, and had a tendency of nonresponse to the concentration change of acid hydrolysate. The characteristic symptom on the presence of peptone that the growth of the main axis tips is less during the earlier period, also disappeared through acid digestion of peptone. The phenomena mentioned above, especially, are marked as compared with the results in the previous paper (3), and may be regarded as the results of decomposition of any beneficial substance contained in peptone with acid digestion.

With alkaline digestion, on the other hand, most of the marked effects of peptone remained in the fresh or dry weight and the thickness of roots, which increased parallel to the amounts of the hydrolysate. The beneficial effects

of peptone, however, partially disappeared with alkaline digestion as seen in the growth of the main axis tips and in the production of laterals. It is to be considered on the facts that the constituents other than the beneficial substances of peptone are possibly digested at the same time, then resulting in a complex reaction to the growth of the excised roots.

Summary

(1) Peptone was fractionated in some constituents with the physical or the chemical preparations, and the effects of each fraction on the growth of the excised wheat roots were observed with the aseptic culture.

(2) If peptone was fractionated with the dialysis through a cellophane membrane, most of the beneficial effects were not kept in the nondiffusible fraction, but were in the diffusible fraction.

(3) Water extracts of dry beer yeast were beneficial for the lateral root growth in the amount of 100 mg per liter, but were markedly inferior to peptone.

(4) B vitamins, *i.e.* thiamin, pyridoxine and nicotinic acid could not be substituted for peptone for the growth of the excised roots, and then it was inferred that the beneficial constituents of peptone are different from these vitamins.

(5) The beneficial materals of peptone appear to be insoluble in ether and almost insoluble in absolute and 80 per cent alcohol, since most of the beneficial effects were not found in the fraction soluble in these solvents.

(6) Separated in some fractions with paper chromatography employing phenol as the developing solvent, the beneficial effects on the growth were not seen in any fractions.

(7) With acid digestion of peptone, the characteristic symptoms of the peptone effects on the root growth mostly disappeared, while with alkaline digestion most of them remained.

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