CCLXXXIV. THE DETERMINATION OF LIGNIN. II. ERRORS INTRODUCED BY THE PRESENCE OF PROTEINS.

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THE methods for the determination of lignin have mainly been developed by wood chemists upon materials which are very low in nitrogenous constituents. When however it is desired to apply these methods to less mature plant materials, containing considerable amounts of protein, it has been found that the isolated lignin residue contains some nitrogen. Paloheimo [1925] recognising this stated that all lignin figures obtained on nitrogenous materials should be corrected by subtracting an amount equal to the nitrogen content of the lignin × 6·25. In other words he made the assumption that the nitrogen in the lignin residue is present as protein. The same correction has been employed by Waksman and Stevens [1930] and by Phillips [1932]. The latter worker, however, admits that this may not be justified but considers that at present it is the best procedure that can be adopted.

The experiments to be described deal with the effect of the presence of protein on the lignin figure as determined by 72 % acid treatment and were designed partly to test the validity of such a correction and partly to seek such conditions as should minimise the disturbance from this source.

EXPERIMENTAL.

(*a*) *The nature and magnitude of the disturbance due to protein.*

If proteins, such as egg-albumin or caseinogen, in a finely divided condition are allowed to stand for 16 hours with 72 % H_2SO_4, and the solution is then diluted to 3 % and boiled, no precipitate is obtained; if they are added to pure cellulose, such as filter-paper, and similarly treated, the precipitate, if any, is negligible. On the other hand, if protein be added to straw and the mixture treated as above, the apparent lignin content of the straw will be increased and the lignin residue will contain some nitrogen. In effect, therefore, the lignin itself, or some other straw constituent, acts as a precipitant for a portion of the nitrogenous material in solution. This may be seen from Table I. All lignin determinations in this and subsequent Tables, unless a statement to the contrary is made, were carried out in quadruplicate, two then being used for the ash correction and two for the estimation of nitrogen.

All determinations made in the presence of added protein filtered more slowly, the rate being roughly proportional to the amount added. The effect of the

Table I. *The addition of caseinogen to oat straw and hydrolysed oat straw (5 % acid, 1 hour).*

Straw 0·52 % N. Hydrolysed straw 0·47 % N. Caseinogen 12·43 % N.
16 hours with 72 % acid, temp; <20°; diluted to 3 % and boiled for 2 hours.

Mixture	Apparent lignin g.	N in lignin mg.	N in straw mg.	N in caseinogen mg.	Total N present mg.	% N recovered	Increment in lignin g.	Increment in lignin / N in lignin
(A) 0·8 g. straw alone	0·1449	2·33	4·16	—	4·16	56	—	—
,, ,, +0·2 g. caseinogen	0·2275	4·25	4·16	24·85	29·01	15	0·0826	43·0
(B) 0·4 g. hydrol. straw alone	0·1059	1·34	1·88	—	1·83	71	—	—
,, ,, +0·2 g. caseinogen	0·1271	5·06	1·88	24·85	26·73	19	0·0222	6·0

presence of protein seemed to be to give a cloudy solution in which the lignin did not settle out but remained partly in suspension. Mixtures containing a high percentage of protein could not be filtered at all. In Table I is given a balance sheet of the nitrogen present initially and recovered in the lignin. Only 15 % remained when 0·2 g. caseinogen was added to the straw and 19 % when it was added to a smaller, but approximately equivalent, amount of hydrolysed straw. In order to throw some light on the factor which should be applied to correct for this nitrogen disturbance, the ratio of the increment produced by the addition of protein to the increase in nitrogen found in the lignin has been calculated. This was widely divergent in the two cases, being much higher in the case of the untreated straw. This suggested the possibility of some interaction between hydrolysable constituents, perhaps pentose, and protein, as a result of which the disturbing effect of both might be mutually enhanced. To test this point a number of mixtures of xylose and caseinogen were set up in quadruplicate and treated as in the lignin determination.

Table II. *Interaction of xylose and protein in production of apparent lignin.*

16 hours with 72 % H_2SO_4, temp. <20°; diluted to 3 % acid and boiled for 2 hours.

Mixture	Apparent lignin g.	N in lignin mg.	N in caseinogen mg.	% N recovered	Increment due to caseinogen g.	Increment / N in lignin
0·5 g. xylose alone	0·0078	—	—	—	—	—
,, ,, +0·01 g. caseinogen	0·0227	0·6	1·24	48	0·0149	24·8
,, ,, +0·025 g. ,,	0·0249	1·5	3·11	48	0·0171	11·4
,, ,, +0·05 g. ,,	0·0181	1·25	6·22	20	0·0103	8·2
,, ,, +0·1 g. ,,	0·0339	5·0	12·43	40	0·0261	5·2
,, ,, +0·15 g. ,,	0·0231	1·4	18·65	7	0·0153	10·9
,, ,, +0·2 g. ,,	0·0026	0·25	24·86	1	—	—
,, ,, +0·25 g. ,,	0·0003	—	31·08	—	—	—

That there is an interaction between pentose and protein is clear, but its nature is rather obscure. At low proportions of protein to pentose the effect is markedly to increase the production of insoluble material from the pentose, so much so that when the rate of xylose to caseinogen was highest the apparent lignin figure was almost trebled and nearly 50 % of the added nitrogen was recovered. The latter is not in proportion to the increment caused, which amounted to more than the caseinogen added. With larger additions of protein, however, the yields of insoluble residue decrease, even below that which would be given by the xylose alone, until the point is reached when there is virtually no precipitate at all. Thus the result of the interaction between pentose and protein seems to be much to enhance the effect of the former at low concen-

tration, of the latter, but at high concentrations to reduce or even to nullify it. The nature of the reaction involved has not been investigated, but no doubt it is concerned with the production of furfuraldehyde from the xylose and its linkage with amino-groups.

This variable interaction makes it extremely difficult, if not impossible, to predict the magnitude of the disturbance to be expected in any particular material. That the same order of diminishing disturbance with increasing protein takes place with natural materials is seen in the first part (A) of Table III. Unfortunately it was not possible to test the effect of a further addition of caseinogen as the filtration was then impossibly slow. With 0·1 g. caseinogen added, the lignin required 1 hour for filtration, with 0·2 g., 1 day, and with 0·3 g., 5 days.

The main purpose of the experiments summarised in Table III was to attempt to distinguish between the protein disturbance alone, that produced by pentose and the interaction of the two. For this purpose the amount of hydrolysed straw taken was exactly equivalent to the untreated straw, it being determined that the loss on hydrolysis was 44·91 %. Inasmuch as in hydrolysed material the pentose content is quite low (the furfuraldehyde yield was reduced by about 85 % by the treatment), the figures in the second part (B) of Table III may be taken to represent the effect of protein alone. The magnitude of the

Table III. *The effect of addition of caseinogen to oat straw and to an equivalent amount of hydrolysed oat straw (5 % acid, 1 hour).*

Conditions as in Table I.

Mixture	Apparent lignin g.	N in lignin mg.	N in straw mg.	N in caseinogen mg.	Total N present mg.	% N recovered	Increment due to caseinogen	Increment / Increment of N in lignin
(A) 0·571 g. straw alone	0·1059	1·65	2·97	—	2·97	55	—	—
,, ,, +0·1 g. caseinogen	0·1245	4·5	2·97	12·43	15·40	29	0·0186	6·5
,, ,, +0·2 g. ,,	0·1361	5·65	2·97	24·85	27·82	20	0·0312	7·8
,, ,, +0·3 g. ,,	0·1264	5·6	2·97	37·28	40·25	14	0·0205	5·2
(B) 0·315 g. hydrol. straw alone	0·0696	0·9	1·48	—	1·48	61	—	—
,, ,, +0·1 g. caseinogen	0·1113	4·1	1·48	12·43	13·91	29	0·0417	13·0
,, ,, +0·2 g. ,,	0·1107	4·75	1·48	24·85	26·33	18	0·0411	10·7
,, ,, +0·3 g. ,,	0·1136	4·45	1·48	37·28	38·76	12	0·0440	12·4
(C) 0·315 g. hydrol. straw +0·2 g. xylose	0·0916	1·15	1·48	—	1·48	78	—	—
,, ,, +0·2 g. xylose +0·1 g. caseinogen	0·1247	5·1	1·48	12·43	13·91	37	0·0331	8·4
0·315 g. hydrol. straw +0·2 g. xylose +0·2 g. caseinogen	0·1278	5·7	1·48	24·85	26·33	22	0·0362	8·0
0·315 g. hydrol. straw +0·2 g. xylose +0·3 g. caseinogen	0·1251	5·5	1·48	37·28	38·76	14	0·0335	7·7

increment produced does not change greatly with 0·1, 0·2, or 0·3 g. of added caseinogen, and as a result the percentage of nitrogen recovered in the lignin decreases. The ratio of increment produced to increase in nitrogen found is higher than it would be if the increment were merely protein in nature. This is in contrast to the rather similar conditions of part (B) in Table I in which the ratio is about 6·0. The conclusion to be drawn from both these experiments is, however, that there is some interaction between protein and lignin in the presence of 72 % acid as a result of which an insoluble product is formed. That combination between lignin and protein can take place has been shown by Hobson and Page [1932] and by Waksman and Iyer [1932]. The product obtained by them is stable and the nitrogen cannot easily be removed. It seems not improbable that the disturbance caused by protein in the lignin determina-

tion is due to the formation of some compound of a similar sort. This combination cannot however be a direct one or else the ratio of the increment produced over nitrogen present would approximate to 6. Because of the variability of this ratio, the assumption is that it is protein degradation products formed by the action of the strong acid, rather than protein itself, which are concerned. The fact that in Table III (B) the increments in apparent lignin did not increase with increasing additions of protein rather suggests that the lignin has a limited combining power with such nitrogenous products, and that this was fully satisfied by the lowest amount added.

Turning now to Table III (A) in which untreated straw was used, three factors are involved, firstly the disturbance due to pentose, secondly that due to protein and thirdly that due to the interaction between pentose and protein, which, as shown earlier, might result in a reduction of the effect of the first factor. The increments due to the addition of caseinogen are not nearly so great as in (B) when the equivalent amount of hydrolysed straw was used, and therefore, though the percentages of nitrogen recovered in the lignin are almost identical, the ratios of the increment over increase of nitrogen recovered are much lower. In this case the figures are of the same order as would be obtained if the increment were solely due to protein, but in view of other experiments this must be accounted a coincidence.

In Table III (C) the same three interacting factors are concerned as in (A), since by addition of xylose to the hydrolysed material the effect of pentose and its interaction with protein are re-introduced. The yields of apparent lignin and the percentage recovery of nitrogen are all very similar to those in (A), as might be expected. The same sparing effect on addition of 0·3 g. caseinogen occurs. The close coincidence of these results on synthetic mixtures with those in part (A) may be taken as evidence that the factors concerned have been correctly distinguished.

In all these experiments described above the percentage of nitrogen recovered in the lignin is highest when small quantities of protein are present. Furthermore, increasing the protein beyond a certain point results actually in a decrease in the amount of apparent lignin formed. This is well illustrated in Table III (A) and (C) when the addition of 0·3 g. caseinogen in both cases gave a lower yield of apparent lignin than 0·2 g. That this fact is probably connected with the presence of pentose is shown by part (B) in which the same sparing effect

Table IV. *The effect of addition of small quantities of caseinogen to oat straw and to an equivalent amount of hydrolysed straw (5 % acid, 1 hour).*

Conditions as in Table I.

Mixture	Apparent lignin g.	N in lignin mg.	N in straw mg.	N in casein-ogen mg.	Total N present mg.	% N re-covered	Increment due to casein-ogen g.	Increment in lignin / Increment of N in lignin
(A) 0·8 g. straw alone	0·1587	2·0	3·04	—	3·04	67	—	—
,, ,, + 25 mg. caseinogen	0·1670	3·2	3·04	3·11	6·15	52	0·0083	6·9
,, ,, + 50 mg. ,,	0·1817	4·6	3·04	6·22	9·28	49	0·0230	8·8
,, ,, + 75 mg. ,,	0·1805	5·3	3·04	9·33	12·37	43	0·0218	6·6
,, ,, +100 mg. ,,	0·1765	5·2	3·04	12·43	15·47	34	0·0178	5·6
,, ,, +150 mg. ,,	0·1876	6·1	3·04	18·65	21·69	28	0·0289	7·0
(B) 0·415 g. hydrol. straw alone	0·1059	1·4	1·62	—	1·62	86	—	—
,, ,, + 25 mg. caseinogen	0·1183	2·45	1·62	3·11	4·73	52	0·0124	11·8
,, ,, + 50 mg. ,,	0·1230	3·0	1·62	6·22	7·84	38	0·0171	10·7
,, ,, + 75 mg. ,,	0·1196	3·25	1·62	9·33	10·95	30	0·0137	7·4
,, ,, +100 mg. ,,	0·1177	3·2	1·62	12·43	14·05	23	0·0118	6·6
,, ,, +150 mg. ,,	0·1112	3·6	1·62	18·65	20·27	18	0·0053	2·4

was not manifest. Some experiments, similar in nature to those described in Table III, were made in which the effect of quite small additions of protein was studied, from 25 to 150 mg. caseinogen being added to 0·8 g. straw and to an equivalent amount of hydrolysed straw. These results are given in Table IV.

In (A) in which caseinogen was added to straw the percentage of nitrogen recovered in the lignin falls steadily, but the apparent lignin yield shows an initial rise, a secondary fall and then a further increase. The reason for this peak at the point when 50 mg. of caseinogen were present is not clear but, inasmuch as it occurs also in (B) in which hydrolysed straw is employed, it is probably connected with the interaction between lignin and protein, rather than a protein-pentose effect. The ratios of increment in lignin to the increase of nitrogen recovered are very variable in (A), all but one being significantly higher than 6, in contrast to (B) in which they fall steadily from 12 to 2·4. With an equivalent amount of hydrolysed straw and increasing amounts of caseinogen the percentage of nitrogen recovered in the lignin falls faster than in (A), demonstrating again that with smaller quantities of protein present there is a pentose-protein reaction resulting in the retention of a part of the nitrogen in an insoluble precipitate. The nature of this reaction is not known, but in view of the observations in the previous paper it is likely that furfuraldehyde is concerned in a condensation with some protein fission product or products.

The addition of a small quantity of furfuraldehyde to caseinogen in the presence of 72 % sulphuric acid resulted in a dark-coloured solution, the intensity varying with the amount of protein present. Only a trace of precipitate was formed on dilution and boiling, but it is perhaps significant that this was greatest in amount when the protein added was low (Table V).

Table V. *The addition of furfuraldehyde to small quantities of caseinogen.*

Conditions as in Table I.

Mixture 0·25 ml. furfuraldehyde with	Weight of precipitate mg.
25 mg. caseinogen	4·2
50 mg. ,,	5·4
75 mg. ,,	4·1
100 mg. ,,	3·3
150 mg. ,,	2·5
200 mg. ,,	1·6

The most practical point arising out of these experiments is the fact that any attempt to calculate the disturbance due to protein and to apply a correction for it is useless in view of the interplay of the various factors involved.

(*b*) *The reduction of the disturbance due to protein.*

Since there appears to be no possible means of calculating from the nitrogen content of the lignin the error due to protein, means were sought by which it could be minimised. The first step was to examine whether the reduction of the time of contact with 72 % acid from 16 hours to 2 hours would effect any reduction in the nitrogen content of the lignin. Employing tares, rich in protein, the comparative figures obtained were:

1 g. tares: 16 hours. Apparent lignin 0·1647 g. containing 9·25 mg. N.
1 g. tares: 2 hours. Apparent lignin 0·1500 g. containing 9·9 mg. N.
1 g. lucerne: 16 hours. Apparent lignin 0·1503 g. containing 6·9 mg. N.
1 g. lucerne: 2 hours. Apparent lignin 0·1500 g. containing 6·3 mg. N.

Similar results were obtained with artificial mixtures of straw and protein, some of the figures being given in Table VI, from which it may be seen that there is relatively little difference in the percentages of nitrogen retained by the lignin.

Table VI. *Effect of time of contact with 72 % acid on the nitrogen in lignin from mixtures of straw and protein (egg-albumin).*

Time with 72 % acid stated, temp. <20°; diluted to 3 % and boiled for 2 hours.

Mixture	Time hours	Apparent lignin g.	N in lignin mg.	N in straw mg.	N in protein mg.	Total N present mg.	% N recovered	Increment due to protein g.	Increment / N in lignin
1 g. straw alone	16	0·1848	2·45	5·2	—	5·2	47	—	—
,, ,,	2	0·1529	2·0	5·2	—	5·2	38	—	—
1 g. straw +0·1 g. protein	16	0·2275	6·65	5·2	11·71	16·91	39	0·0427	10·2
,, ,,	2	0·2246	6·1	5·2	11·71	16·91	36	0·0717	17·5
1 g. straw +0·25 g. protein	16	0·2482	9·5	5·2	29·27	34·47	27	0·0634	9·0
,, ,,	2	0·2006	7·55	5·2	29·27	34·47	22	0·0477	8·6
0·6 g. hydrol. straw alone	16	0·1327	1·7	2·8	—	2·8	61	—	—
,, ,,	2	0·1334	1·75	2·8	—	2·8	62	—	—
0·6 g. hydrol. straw +0·25 g. protein	16	0·1638	6·2	2·8	29·27	32·07	19	0·0311	6·9
,, ,, ,,	2	0·1760	6·75	2·8	29·27	32·07	21	0·0426	8·5

These and many other observations make it clear that the shorter period has no advantage as far as the nitrogen content of the product is concerned. To lower this, recourse must be had apparently to some pretreatment which reduces the nitrogen content of the original material without affecting the lignin itself. Even so, unless the removal of nitrogenous constituents be very complete little would be gained, since, as seen repeatedly in the previous section, the lignin disturbance is highest when small quantities of protein are present.

Attempts were made to remove the protein by a pepsin digest, alone, and followed by boiling for 1 hour with 5 % H_2SO_4. 1 g. tares was suspended in 25 ml. 0·1 *N* HCl and, after the addition of 2 ml. of a 5 % pepsin solution, kept at 40°. The yields of lignin and its nitrogen content after this pretreatment are given in Table VII.

Table VII. *Attempted removal of protein by pepsin and acid hydrolysis.*

16 hours with 72 % H_2SO_4, temp. <20°; diluted to 3 % and boiled for 2 hours.

1 g. material treated in various ways	Residue g.	Apparent lignin g.	N in lignin mg.
Untreated	1·000	0·1647	9·25
Digested with pepsin— 3 hours	0·640	0·1391	6·35
Digested with pepsin—40 hours	0·566	0·1187	4·5
Digested with pepsin—3 hours, then hydrolysed 1 hour with 5 % acid	0·388	0·1122	4·5
Digested with pepsin—40 hours, then hydrolysed 1 hour with 5 % acid	0·368	0·1021	3·35
Hydrolysed 1 hour with 5 % acid	0·400	0·1177	5·4

Though the disturbance due to protein is much lowered by pepsin digestion, it is not markedly less than when a simple acid hydrolysis alone is given. Any biological treatment to be adopted, because of the time consumed, must show very definite advantages over a chemical treatment. Neither pepsin digestion nor fermentation with highly active protein-decomposing organisms, as tried subsequently, fulfilled this requirement. Of possible chemical treatments, alka-

line extraction, which would be the most effective, is ruled out because of its solvent action on lignin. In Table VIII is given the result of removing nitrogen from lucerne by various means.

Table VIII. *Removal of nitrogen from lucerne (original N = 2·75 %).*

Nitrogen expressed as percentage of the original N content.

Treatment	% N recovered
Water at 100°—½ hour	60·2
NaCl 10 % at 40°—2 hours	48·7
H_2SO_4 5 % at 100°—1 hour	19·7
H_2SO_4 5 % at 100°—5 hours	13·1
Pepsin at 40°—3 days	19·0
Takadiastase at 40°—3 days	62·5

The most suitable treatment is acid hydrolysis for 1 hour with 5 % H_2SO_4, the same procedure as was employed in previous work for the reduction of the disturbance due to pentose. It is still subject to the criticism that it has not been unquestionably shown that lignin is unaffected by dilute acid prior to contact with 72 % H_2SO_4. Further, since the acid pretreatment does not completely remove protein, but only results in a lowering of the protein content, this expedient is not particularly satisfactory in the case of materials originally high in nitrogen. Indeed one or two cases have occurred in which the nitrogen in the lignin has been a little higher after such hydrolytic pretreatment. Examples of some green materials are given in Table IX.

Table IX. *Effect of hydrolytic pretreatment in lowering the nitrogen in lignin from certain green materials.*

All results expressed on 1 g. original material.

Material	N in material mg.	Apparent lignin mg.	N in lignin mg.	Recovery of N %
Kale 1 g.	42·8	53·5	2·0	5
Hydrolysed kale 0·279 g.	3·8	58·2	2·0	53
Maize 1 g.	27·6	182·4	9·6	35
Hydrolysed maize 0·365 g.	7·7	79·9	3·6	47
Bean 1 g.	34·2	150·2	5·2	15
Hydrolysed bean 0·372 g.	9·1	117·1	5·4	60
Grass 1 g.	32·4	179·3	10·3	32
Hydrolysed grass 0·414 g.	12·2	132·9	6·5	53
Wheat 1 g.	13·1	213·0	5·7	44
Hydrolysed wheat 0·504 g.	4·9	128·0	2·4	49

In two out of the five materials taken, the nitrogen in the lignin was almost unaffected by the hydrolytic pretreatment, while in the remaining three cases a considerable reduction was effected.

It was shown earlier that a shorter period of contact with the 72 % sulphuric acid did not result in any lowering of the nitrogen in lignin. This is also the case if the full procedure of Ritter *et al.* [1932] be used. Comparisons of this method with direct determinations are given in Table X.

Hydrolytic pretreatment, therefore, appears to be only partially successful

Table X. *Comparison of nitrogen in lignin obtained by Ritter-Seborg-Mitchell method with direct determinations.*

All results expressed on a basis of 1 g. original material.

		16 hours with acid		2 hours with acid		R.-S.-M. method		16 hours (after hydrolysis)	
Material	N content mg.	Apparent lignin g.	N in lignin mg.	Apparent lignin g.	N in lignin mg.	Apparent lignin g.	N in lignin mg.	Apparent lignin g.	N in lignin mg.
Lucerne	35·4	0·1503	6·9	0·1500	6·3	0·1510	7·9	0·1460	5·8
Rotted rice straw	5·2	0·1082	2·8	0·1194	2·2	0·1045	2·6	0·0887	2·3
Rotted rye straw	32·9	0·4680	13·8	0·4945	17·5	0·4403	14·9	0·4195	9·7
Rotted oat straw	18·6	0·3085	7·5	0·3010	7·8	0·2420	6·7	0·2565	5·2

in reducing the protein disturbance, but no other expedient either in the nature of a pretreatment or a modification of conditions achieves as much. Until an alternative is found, this treatment will be given, inasmuch as it minimises the effect of both protein and pentose. On theoretical grounds it should then be immaterial whether the time of contact with 72 % acid is 2 hours or 16 hours, but since with certain materials high in nitrogen the shorter periods caused a higher yield of apparent lignin and a higher content of nitrogen in lignin, the 16-hour procedure is preferable. In any case the nitrogen content of the lignin should be determined and recorded but no attempt made to apply any correction by calculating as protein and subtracting from the apparent lignin yield. Sufficient evidence has been given to indicate that this procedure may introduce errors larger than those which it is designed to correct.

Summary.

1. Proteins alone give no precipitate on standing with 72 % H_2SO_4 but when added to plant materials increase the apparent lignin content. The lignin residue then obtained contains nitrogen.

2. The magnitude of the disturbance produced is quite different if the material is previously subjected to a hydrolytic pretreatment, thus indicating some interaction between the hydrolysable constituents and protein, which enhances the disturbing effect of both.

3. If xylose and protein are treated together with 72 % acid, insoluble precipitates are formed when the protein present is small in amount. Increasing quantities of protein give diminishing yields of precipitate, till none is formed. This sparing effect of larger quantities of protein has also been observed with plant materials.

4. By comparing the effects of additions of protein to untreated straws and to an equivalent amount of hydrolysed straw, it is possible to distinguish between the protein disturbance alone, that produced by pentose and that produced by the interaction of the two. Small additions of protein cause a proportionately greater disturbance than do larger amounts.

5. The protein disturbance is probably due to the linkage of protein fission products with lignin. Direct linkage between protein and lignin is unlikely because the ratio of increment produced to nitrogen present is very variable. To apply a correction by calculating the nitrogen in the lignin as protein and subtracting is useless and likely to introduce in some cases an error greater than that caused by the presence of nitrogenous material.

6. The magnitude of the disturbance cannot be reduced by decreasing the time of contact with acid from 16 to 2 hours or by following the Ritter-Seborg-Mitchell procedure.

7. Acid pretreatment results in a lowering of the error in most cases and though only partially successful has been provisionally adopted.

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