

# THE CONSTANT OCCURRENCE OF NONREDUCING DISACCHARIDES IN HYDROLYZED INULIN

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## ABSTRACT

It was demonstrated in a previous article that inulin from dahlia tubers yielded upon hydrolysis in aqueous solution with sulphuric acid a sugar mixture consisting of 91.8 per cent fructose, 3 per cent glucose, and 5.2 per cent of a group of nonreducing difructose anhydrides. It is now shown that inulin from wild chicory, dandelions, burdock, goldenrod, and Jerusalem artichoke yields the same percentage of these disaccharides, and that extensive fractional crystallization fails to alter this percentage. Since polysaccharides related to inulin do not yield the disaccharides it is concluded that the latter are an integral part of the inulin molecule and are not produced by side reactions occurring during hydrolysis. The occurrence of the disaccharides in such constant proportions regardless of the source of the inulin or its degree of purification indicates that inulin is essentially a homogeneous substance. Evidence to be presented in a later article suggests that there are at least three different disaccharides in the nonreducing portion of hydrolyzed inulin from which it follows that the molecular weight of inulin may be as high as 18,000.

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## I. INTRODUCTION

In a previous article Jackson and Goergen<sup>1</sup> showed that purified dahlia inulin yielded upon hydrolysis with aqueous acids a sugar mixture consisting of 91.8 per cent fructose, 3 per cent aldose, and 5.2 per cent of a group of nonreducing disaccharides which were of such high stability as to survive the hydrolysis. It was demonstrated that these disaccharides were dextrorotatory and were hydrolyzed at high temperature and acidity solely to fructose. One of them was obtained in crystalline form and was found to be a difructose anhydride of  $[\alpha]_D^{20} = +27$ . The aldose in inulin has been shown by Schlubach and Elsner<sup>2</sup> to be glucose.

<sup>1</sup> B. S. Jour. Research, 3, p. 27 (RP79); 1929.

<sup>2</sup> Ber., 62, p. 1497; 1929.

In continuation of the investigation the present authors have obtained evidence which will be presented in a later article that no less than three different disaccharides are contained in the nonreducing portion of hydrolyzed inulin.

There are two conceptions of the nature of inulin, between which it is the purpose of the present article to distinguish. (1) Inulin may be composed of similarly constructed molecules, each of which contains the refractory difructose residues. Since these residues constitute but 5 per cent of the inulin, the whole molecule must be 20 times as great as the residue itself, and since the residue is composed of at least three different disaccharides, each consisting of two fructose residues, it follows that inulin would under this conception have a very high molecular weight. (2) If a relatively low molecular weight is to be ascribed to inulin it must follow from simple arithmetical considerations that the nonreducing disaccharides will be unequally distributed among the molecules. Assume, for example, that the molecule of inulin is composed of nine fructose residues.<sup>3</sup> If 5 per cent of inulin consists of three disaccharides, each having two fructose residues, then but 1 molecule in 12 can contain the refractory disaccharides if all of the latter are in the same molecule, or 3 in 12, if they are distributed among three molecules.

The distinction between these hypotheses is sharp. If the first is correct all samples of inulin should contain approximately the same per cent of the nonreducing disaccharides. If the second hypothesis is true, it should be possible to obtain samples of inulin from which the molecules containing the disaccharides have been isolated or eliminated, and great variations in the disaccharide content should be observable.<sup>4</sup>

In distinguishing between these hypotheses we shall use as a criterion the constancy with which the nonreducing disaccharides occur in the products of hydrolysis of inulin prepared by a variety of methods and from various sources. This criterion does not require an exact agreement in analytical data and indeed an accurate agreement is not to be expected, since inulin itself shows variations in physical properties and hydrolyzed inulin shows small variations in analytically determined constituents.

If inulin prepared from dahlias is the mixture of polysaccharides assumed under our second hypothesis, it must consist of a number of molecular species which are present in constant proportions in all samples, since it always yields upon hydrolysis approximately 5 per cent of unhydrolyzed disaccharides. It is necessary to assume that these various molecular species exist in the ratio of their solubilities, since their proportions are not altered by recrystallization. In other words, in order to explain the presence of the refractory difructoses in a heterogeneous inulin, we must assume that it is composed of dissimilar polysaccharides of similar solubility. If this be true we should expect that inulin prepared from other plants than the dahlia would show some differences in composition, since, in general, the relative amounts of the different polysaccharides vary from plant to

<sup>3</sup> Pringsheim and Aronowsky, *Ber.*, 55, p. 1414; 1922.

<sup>4</sup> The segregation, of course, could not be effected if this hypothetical mixture were composed of constituents which crystallized in a constant ratio. It will appear in the following pages that such a special case could exist only if the carbohydrates of this mixture were formed in the juices of a number of widely differing plants in exactly the constant crystallizing ratio, a condition so highly improbable as to be negligible.

plant. For example, in the dahlia tuber inulin predominates over the more soluble polysaccharides, while in the Jerusalem artichoke, as Tanret<sup>5</sup> has shown, the polysaccharide, synanthrin, predominates, although inulin is also present in relatively small proportion. It is therefore pertinent to examine inulin prepared from other plants in order to determine whether variations in the disaccharide content occur.

## II. PREPARATION AND ANALYSIS OF INULIN

In pursuance of this plan we have prepared inulin from the roots of burdock, goldenrod, dandelion, artichoke, and wild chicory. All samples were gathered during the months of October and November in the vicinity of the National Bureau of Standards, except one sample of burdock which was gathered in Wisconsin.<sup>6</sup> All the plants mentioned belong to the family of *Compositae*. It is doubtful if true inulin occurs outside of this family. Each sample of inulin was recrystallized at least twice and finally hydrolyzed and analyzed in the manner described in the previous article.

The analytical results are assembled in Table 1 (a). It is at once evident from column 4 that the total reducing sugar content of all samples of hydrolyzed inulin is approximately the same and in average amounts to 94.7 per cent. The nonreducing residue is therefore 5.3 per cent, whereas dahlia inulin from the previous investigation showed 5.2 per cent. (Table 1 (c).) This approximate constancy of composition of inulin derived from such a wide variety of sources affords strong evidence that inulin is not the heterogeneous mixture postulated under our second hypothesis. On the other hand, the experimental data are reasonably interpreted by our first hypothesis and we may draw the conclusion that every molecule of inulin contains about 5 per cent of these nonreducing refractory disaccharides.

The glucose content of the inulin from the miscellaneous plants amounts to 3.7 per cent. This is slightly higher than the 3.0 per cent in dahlia inulin. Similarly the fructose content of 91.0 per cent is slightly lower than in dahlia inulin which was previously found to be in average 91.8 per cent. The higher glucose and lower fructose contents are also reflected in the slightly lower specific rotation of the products of hydrolysis.

<sup>5</sup> Bull. Soc. Chim., 3d series, 9, pp. 227, 622; 1893.

<sup>6</sup> Very kindly submitted by Miss Norma McDonald.



TABLE 1.—Summary of analyses

## (a) INULIN FROM MISCELLANEOUS PLANTS

	Source of inulin	Number of crystallizations	Fructose	Total reducing sugar	Glucose by difference	Ratio: Fructose to reducing sugar	Difructose residue	$[\alpha]_D^{20}$ after hydrolysis	$[\alpha]_D^{20}$ residue
			Per cent	Per cent	Per cent	Per cent	Per cent		
1	Wild chicory.....	2	91.3	94.9	3.6	0.962	5.1	-80.4	+47
2	Burdock.....	2	90.1	93.7	3.6	.962	6.3	-78.5	+52
3	do.....	2	89.1	94.5	5.4	.942	5.5	-78.4	+21
4	do.....	3	91.4	94.9	3.5	.963	5.1	-80.7	+49
5	Dandelion.....	2	91.8	95.0	3.2	.967	5.0	-81.4	+41
6	do.....	3	90.7	95.1	4.4	.954	4.9	-79.2	+51
7	Goldenrod.....	3	91.7	94.7	3.0	.969	5.3	-80.7	+42
8	Artichoke.....	2	90.6	94.2	3.6	.963	5.8	-78.7	+56
9	do.....	4	92.0	95.0	3.0	.968	5.0	-80.8	+56
	Average.....		91.0	94.7	3.7	.961	5.3	-79.9	+49

## (b) FRACTIONATION OF INULIN

10	Artichoke.....	11	91.3	94.4	3.1	0.967	5.6	-80.3	+52
11	Dahlia.....	11	92.5	94.4	1.9	.980	5.6	-80.8	+50

(c) DATA OF JACKSON AND GOERGEN<sup>2</sup>

	Dahlia.....		91.8	94.8	3.0	0.969	5.2	-82.6	
	Average all samples.....		91.4	94.7	3.3	.966	5.3	-81.1	

<sup>1</sup> Not included in average.<sup>2</sup> Average of eight analyses.<sup>3</sup> The rotations were measured by Jackson and Goergen, but were inadvertently omitted from their article.

Inulin-bearing plants contain a series of polysaccharides of varying complexity which culminates in inulin itself. As we ascend the series we find a diminishing ratio of glucose to fructose which reaches its minimum in inulin. In the dahlia the development of the more complex members of the series has proceeded to such an extent that inulin is the predominating polysaccharide. We should, therefore, expect a more nearly complete elimination of glucose in the dahlia inulin than in related plants which appear to have less ability to synthesize inulin.

It requires to be shown that the nonreducing residue from the inulin from the miscellaneous plants has the same composition as that from the dahlia inulin. To have isolated the residue from each small sample of hydrolyzed inulin and to have determined its properties would have been a prohibitive labor. We have, therefore, endeavored to determine its specific rotation by subtracting the algebraic sum of the rotations of the known constituents from the observed rotation. The results are calculated from small differences between large numbers, and hence errors are multiplied about twenty-fold. Nevertheless, the results of this calculation, which are shown in the last column of Table 1, indicate that the residues have dextrorotatory powers of the same order of magnitude as the residue from hydrolyzed dahlia inulin which Jackson and Goergen found to be about +55.

## III. FRACTIONATION OF INULIN

In order further to distinguish between our two hypotheses we have subjected inulin to a fractional crystallization in an effort to isolate fractions containing higher proportions of the stable difructose residues. If the inulin molecule consisted of the six or nine fructose residues usually ascribed to it, there would be some molecules in which the disaccharides constituted as much as 20 or more per cent and other molecules from which the disaccharides were absent. Such a difference in composition would certainly result in a difference in solubility. We have, therefore, subjected samples of dahlia inulin and artichoke inulin to 11 fractionations, rejecting the mother liquor after each operation. The conditions were so chosen that 85 per cent of the dahlia inulin was rejected in the mother liquor and 96 per cent of the artichoke inulin. The final fractions, consisting, respectively, of but 15 and 4 per cent of the initial material, were then hydrolyzed and analyzed as usual. The analytical data shown in Table 1 (b) indicate that these final fractions have essentially the same composition as the initial material. After so many operations and such lavish rejection of the more soluble fractions any nonhomogeneity must have manifested itself by some change in composition between the original and final fractions.

The decreased content of glucose in the dahlia inulin is of interest. It is another instance of the fact shown by Tanret that in the series of fructose polysaccharides a diminution in glucose content is accompanied by a decrease in solubility. Apparently in inulin itself some molecules contain more glucose than others and would thus have a higher solubility. These more soluble molecules have been eliminated by our fractionation. That the artichoke inulin does not show so great a decrease in glucose content may be explained by the fact that inulin of low glucose content is not formed by the artichoke plant. It has less ability to eliminate glucose from its polysaccharides, as is shown by the great preponderance of synanthrin and other polysaccharides of high glucose content.

## IV. HYDROLYSIS OF RELATED POLYSACCHARIDES

In the article by Jackson and Goergen the suggestion was made that the nonreducing difructose anhydrides might be formed during the hydrolysis by condensation of difructose fragments which might have a momentary existence. Conceivably inulin upon hydrolysis would be ruptured to form a great variety of intermediate products, among which there would be many groups of two fructose molecules which would be capable of either hydrolysis to fructose or condensation to difructose anhydride, the amount of the latter formed depending upon the relative rates of reaction. If such were the case it would explain the constancy of the yield of the nonreducing disaccharides. Militating against this theory is the fact that Jackson and Goergen obtained the same quantity of disaccharides regardless of changed conditions of hydrolysis which might be expected to alter the relative rates of hydrolysis and condensation.

The question can be definitely settled by hydrolyzing related polysaccharides and ascertaining whether they too yield the same 5 per cent of nonreducing residue. We have, therefore, extracted by Tan-



ret's procedure a fraction of the soluble polysaccharides of the Jerusalem artichoke which was soluble in 70 per cent alcohol and a fraction of the polysaccharides in *Iris hookeri* soluble in 60 per cent alcohol. No attempt was made to prepare these substances in a highly purified state, since it was sufficient for the present purpose to learn whether or not they would yield the 5 per cent residue which we found invariably in inulin. That both of these polysaccharides are closely related to inulin is evident from their high fructose content and from their ready hydrolysis under conditions which are adequate for the hydrolysis of inulin. As shown in Table 2, in neither case is there formed the 5 per cent of nonreducing substance which is so characteristic of inulin. The relatively small departure from 100 per cent must be ascribed to analytical uncertainty. In the first analysis the low concentration of substance made the determination of dry substance difficult. The iris polysaccharide yielded apparently 1.4 per cent of nonreducing substance. The specific rotation of this residue was calculated in the manner described for the inulin residues and a very high negative rotation was found, indicating that the residue bore no resemblance to the positively rotating inulin residues. We may, therefore, conclude that the difructose anhydrides are not by-products of the hydrolysis reaction but are true constituents of inulin.

TABLE 2.—Hydrolysis of related polysaccharides

Source	Solvent	Fructose	Total reducing sugar	Glucose by difference	$[\alpha]_D^{20}$ after hydrolysis
Artichoke.....	70 per cent alcohol.....	Per cent 84.5	Per cent 100.7	Per cent 16.2	—70.2
Iris.....	60 per cent alcohol.....	96.0	98.6	1.6	—83.3

## V. THE MOLECULAR WEIGHT OF INULIN

If the foregoing demonstration and its interpretation are valid we are enabled to draw some tentative conclusions in regard to the magnitude of the inulin molecule. The final solution of this question must necessarily await the resolution of the disaccharide residue into its constituent parts in order to determine the number and relative amounts of the different difructoses contained in it. Evidence which will be presented in a later article leads us to the belief that there are three difructoses in the residue. For the present discussion we shall assume that these three are present in equal amounts.

For the composition of inulin we have taken an average value (Table 1) of all samples analyzed, including the seven analyses of Jackson and Goergen. The average difructose content is 5.30 per cent. We have made the tentative assumption that this is made up of three different compounds, each of which is therefore 1.77 per cent of the whole inulin molecule. Each of these compounds contains two fructose residues; hence, one fructose residue is 0.89 per cent of the molecule. The inulin molecule must then have the following constitution:

	Difruc- tose I	Difruc- tose II	Difruc- tose III	Glucose	Fructose
Residue, per cent.....	1.77	1.77	1.77	3.25	91.45
Number of hexose residues.....	2	2	2	3 or 4	102

The whole molecule must then contain not less than 111 hexose residues which, upon condensation and loss of 110 molecules of water, would produce a polysaccharide having a molecular weight of about 18,000.

The possibility must not be overlooked that these disaccharides may have occurred in the inulin molecule as a single residue and that their isomerism may have been caused by different modes of cleavage from the rest of the molecule. As we know nothing of the structure of the difructoses, the mechanism of such a reaction can not be suggested. If such were the case, however, the molecular weight of inulin would fall to about 6,000, in fair agreement with the cryoscopic determination made by Tanret. It is, however, not our purpose to emphasize the quantitative interpretation of these data. We desire merely to indicate that inulin has a high rather than a low molecular weight.

The highest previously estimated value of the molecular weight is that determined by Tanret <sup>7</sup> who found by the cryoscopic method a value of 4,827. Since one hexose residue, set free by hydrolysis, would diminish the apparent molecular weight by 50 per cent, Tanret's measurement must be considered a minimum value, for the important sources of error tend toward low rather than high values.

Pringsheim and Aronowsky <sup>8</sup> found that triacetyl inulin in acetic acid possessed a molecular weight corresponding to nine fructose residues. Schmidt and Becker <sup>9</sup> and Reihlen and Nestle <sup>10</sup> by cryoscopic and tensimetric methods, respectively, found a molecular weight of inulin in liquid ammonia corresponding to a disaccharide. Bergmann and Knehe <sup>11</sup> obtained a similar result from a cryoscopic determination of the molecular weight of inulin acetate in glacial acetic acid. The latter authors conclude that inulin under these conditions is dissociated into difructose units. Recently Berner <sup>12</sup> has demonstrated that inulin adsorbs alcohol and other organic solvents and retains them tenaciously. He concludes that many of the abnormally low molecular weight determinations previously reported, as well as the evidences of depolymerization are vitiated by the errors caused by the depressions of freezing points due to adsorbed solvent. After applying corrections for these influences he found values for the molecular weight of inulin of about 3,500. This latter value is in agreement with the ebullioscopic determination of Drew and Haworth <sup>13</sup> who concluded that the inulin molecule contained from 20 to 24 fructofuranose rings.

In ascribing a value to the molecular weight of inulin certain qualitative facts must be considered. In the analyses described in the present paper relatively considerable variations in the glucose and fructose contents are found. These variations occurred without perceptible variations in the physical properties of the inulin. The most

<sup>7</sup> See footnote 5, p. 1153.

<sup>8</sup> Ber., 54, p. 1281; 1921; 55, p. 1414; 1922.

<sup>9</sup> Ber., 58, 1968; 1925.

<sup>10</sup> Ber., 59, 1159; 1926.

<sup>11</sup> Ann., 449, p. 302; 1926.

<sup>12</sup> Ber., 63, p. 1356; 1930.

<sup>13</sup> J. Chem. Soc., p. 2690; 1928.



probable interpretation is that they were small variations in the number of hexose residues in a molecule of high molecular weight.

Moreover, we must also consider the remaining members of the polysaccharide group. There is a series of these substances exhibiting very great differences in solubility, many of which are probably free from the refractory disaccharides. If inulin itself had a low molecular weight, it would be difficult to accommodate these lower polysaccharides in a series and account for the striking differences in physical properties.

## VI. CONCLUSION

Inulin is a polysaccharide containing in condensed form about 92 per cent fructose, 3 per cent glucose, and 5 per cent of a group of refractory difructose anhydrides. This group of disaccharides is invariably a constituent of inulin regardless of the plant from which the inulin is extracted or of the degree of purification to which the inulin is subjected. Extensive fractional crystallization fails to alter appreciably the disaccharide content. Since polysaccharides related to inulin do not yield the nonreducing disaccharides the latter can not be a by-product of the hydrolysis reaction. They are, therefore, an integral part of the inulin molecule.

It appears probable from experiments as yet incomplete that there are at least three disaccharides present in the residue from hydrolyzed inulin from which it follows that the molecular weight of inulin may be as high as 18,000. When the composition of the disaccharide mixture is definitely known, it may be expected to serve as a fairly exact unit of measurement to determine the magnitude of the inulin molecule.

The invariable presence of small amounts of glucose suggests that glucose is a part of the inulin molecule. It is probably not formed by rearrangement during hydrolysis, since it is variable in amount and since it is possible partially to eliminate it by fractionation as is shown in analysis No. 11, Table 1.

## VII. EXPERIMENTAL

### 1. EXTRACTION AND HYDROLYSIS OF INULIN

The comminuted roots of burdock, chicory, goldenrod, dandelion, and Jerusalem artichoke were digested with water for about an hour at 70° C., and the juice expressed by a powerful tincture press. To the warmed juice normal lead acetate was added and the filtrate freed from excess lead with hydrogen sulphide. The clear solution was evaporated in a vacuum to about 20 per cent solids and set aside to permit separation of inulin. The recovered inulin was recrystallized one or more times from aqueous solutions heated to about 80° C.

The final product was dissolved in hot water forming a solution of about 13 per cent concentration and hydrolyzed for three hours at 60° C. in the presence of 0.08 *N* H<sub>2</sub>SO<sub>4</sub>. In some instances the temperature was raised to 70° C., but the increased temperature was without appreciable influence on the analytical data. The solution of hydrolyzed inulin was exactly neutralized with recrystallized Ba(OH)<sub>2</sub> in the presence of bromthymol blue and the filtrate subjected to careful analysis for total solids, total reducing sugars, and fructose.



## 2. ANALYSIS OF HYDROLYZED SOLUTIONS

The analysis of the hydrolyzed solutions was conducted in essentially the manner described in the article by Jackson and Goergen. In the present investigation total solids were determined by density measurements at 20° C. and by refractive indices measured in an immersion refractometer. Density and refractive index measurements were made with pure fructose solutions during the course of a parallel research and all dry substance determinations of hydrolyzed inulin solutions were referred to the fructose table. This procedure involved a small systematic error, since only 91 per cent of the dry substance was fructose. As all the measurements in this and the previous article contain this same systematic error the results are strictly comparable.

Total reducing sugar was determined by triplicate titrations by the method of Lane and Eynon.

Fructose was estimated as in the previous research by triplicate analyses by the method of Nyns. The precision of analysis was, however, greatly increased, partly by a slight modification of procedure and partly by the improved technique resulting from experience.

## 3. FRACTIONATION OF INULIN

One hundred grams of dahlia inulin was dissolved in hot water, clarified by carbon, and the filtrate transferred to the aluminum cups of a centrifugal sedimentation machine. The inulin solution was allowed to remain at room temperature until a considerable portion had crystallized and was then sedimented for about one hour at high speed. The mother liquor was poured off and the cups were filled with boiling water to redissolve the inulin. This process was repeated ten times. The final crystals were dissolved in hot water, filtered, and recrystallized. It was found that but 15 g of inulin remained for analysis.

Similarly 34 g of artichoke inulin was fractionated in the same manner and but 1.35 g was recovered in the final fraction for analysis.

## 4. DENSITY AND REFRACTIVE INDEX OF A DIFRUCTOSE ANHYDRIDE SOLUTION

In the calculation of dry substance from density and refractive index measurements it has been assumed that the solids were fructose. In order to determine the magnitude of the error involved we have made measurements on a sample of crystalline difructose anhydride obtained by Jackson and Goergen. Substance, 1.0570 (vacuum); solution, 17.292 (vacuum); volume at 20° C., 16.941; per cent, 6.1127;  $D_4^{20}$ , 1.02074. The same solution gave a reading of 36.64 on the immersion refractometer ( $H_2O = 14.50$ ), whence  $n_D^{20} = 1.34148$ .

A solution of  $D_4^{20} = 1.02074$  would contain 6.113 per cent of difructose anhydride, or 5.671 per cent fructose. The ratio of these percentages is 1.078. The true difructose content of the hydrolyzed solutions is therefore about 7.8 per cent higher than the quantities estimated.

Similarly a solution reading 34.64 in the immersion refractometer would contain 6.113 per cent of difructose anhydride, or 5.871 per cent of fructose. The ratio of these quantities is 1.041 and the true difructose content of the hydrolyzed solutions is about 4.1 per cent higher than the amounts estimated.

## 5. CALCULATION OF SPECIFIC ROTATION OF NONREDUCING RESIDUE

The method of calculating the rotatory power of the nonreducing residue can best be illustrated by a specific example. For example, experiment No. 8:

Dry substance in 100 ml.....	10.809
Nonreducing residue, $10.809 \times 5.83$ per cent.....	0.630
Rotation of fructose, $90.65$ per cent $\times 10.809 \times (-5.382)$ .....	-52.73
Rotation of glucose, $3.52$ per cent $\times 10.809 \times (+3.10)$ .....	+1.57
Rotation of determined constituents.....	-51.16
Observed rotation.....	-49.12
Rotation of residue.....	+2.04

$$\left[ \alpha \right] \frac{20}{D} = \frac{+2.04 \times 0.3462}{0.00630 \times 2} = +56.$$

The rotations were measured in a 2 dm tube on a saccharimeter. The specific rotation of fructose is a function of its concentration and the values for the concentrations of the present analyses were determined during a parallel research which will be described in a later article.

## VIII. SUMMARY

Pure inulin has been extracted from wild chicory, dandelion, golden-rod, burdock, and Jerusalem artichoke. All samples yielded upon hydrolysis with sulphuric acid approximately 91.0 per cent fructose, 3.7 per cent glucose, and 5.3 per cent of the same group of nonreducing difructoses which was previously found in inulin prepared from dahlia tubers.

Extensive fractional crystallization failed to alter essentially the relative proportions of these constituents.

Related polysaccharides gave no indication of the presence of the nonreducing disaccharides.

It is concluded that the refractory difructose anhydrides are an integral part of the inulin molecule and that the molecular weight of inulin may be as high as 18,000.

WASHINGTON, July 8, 1930.