

STUDIES ON OXIDIZED WOOL

III. ION-EXCHANGE AND ACID-UPTAKE CHARACTERISTICS

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[Manuscript received May 1, 1959]

Summary

The acid-uptake and ion-exchange characteristics of wool oxidized with peracetic acid have been re-examined. It has been shown that oxidized wool is capable of ion exchange although the ion-exchange properties are similar to those of carboxylic acid cation-exchange resins and unlike those of sulphonic acid cation-exchange resins. Oxidized wool was successfully converted to the ammonium "salt" and the ammonium ions exchanged with barium ions.

Oxidized wool takes up very little acid even at pH 0.45, which is evidence that the sulphonic acid groups are largely ionized at this pH. It will, however, take up 740 μ -equiv. of alkali per g of dry oxidized wool on titration to pH 5.7 and it is concluded that in water-washed oxidized wool the carboxyl groups are substantially unionized whereas sulphonic acid groups are ionized and internally compensated by the ionized basic groups.

I. INTRODUCTION

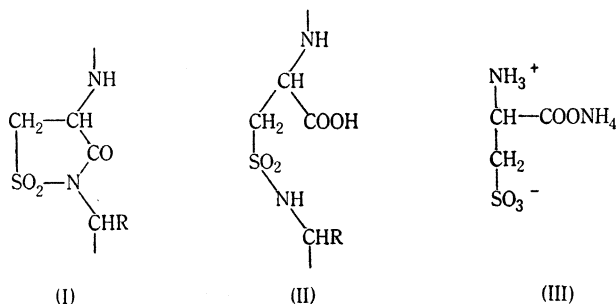
The properties of oxidized wool prepared by treatment of wool with 1.6 per cent. aqueous peracetic acid were studied by Alexander, Fox, and Hudson (1951). It did not liberate acid when treated with potassium chloride, and after equilibration with ammonium chloride no cation exchange occurred when it was subsequently treated with potassium chloride. If strongly acid sulphonic acid groups were present in oxidized wool it would be expected that they would allow ion exchange to occur under the above conditions. To explain this behaviour Alexander, Fox, and Hudson postulated that in oxidized wool there were no sulphonic acid groups but that intramolecular cyclization occurred to form a sulphonimide grouping (I) which would hydrolyse directly to cysteic acid. However, on treatment with dilute ammonia it was postulated that a sulphonamide type of grouping (II) was formed which would be hydrolysed to cysteic acid only with difficulty.

The fact that other oxidized proteins such as oxidized insulin (Sanger 1949*a*, 1949*b*) were known to contain sulphonic acid groups suggested that the explanation of Alexander, Fox, and Hudson is incorrect. Weston (1955) studied the infra-red spectrum of oxidized wool and demonstrated conclusively that $-\text{SO}_3^-$ or SO_3H groups were present. Earland and Knight (1955) explained the absence of the expected ion-exchange properties on the assumption that the $-\text{SO}_3^-$ groups were bound in "salt linkage" to the basic groups. However, if this is the case there should be excess carboxyl groups capable of ion exchange. Earland and Knight

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reported that α -keratose, the acid-precipitable fraction of the alkali-soluble constituents of oxidized wool, did not form an ammonium salt, a fact which, if true, is not explicable on their theory, since the carboxyl groups should form an ammonium salt as is the case with cysteic acid (III).

The experiments reported in this paper on the ion-exchange and acid-uptake properties of oxidized wool support the view that $-\text{SO}_3^-$ groups are present. The ion-exchange properties are those of carboxylic acid groups and are similar to those of the carboxylic acid type of cation-exchange resins, the behaviour of which is very different from that of the sulphonic acid type of ion-exchange resin (Kunin and Barry 1949).



II. MATERIALS AND METHODS

The Merino 64's wool used was that described in Part I of the series (Thompson and O'Donnell 1959). Wool oxidized for 24 hr with 1.6 per cent. aqueous peracetic acid as described by O'Donnell and Thompson (1959) was used in all experiments.

Measurements of pH were made with a glass electrode standardized at pH 4 with 0.05M potassium hydrogen phthalate. Automatic titrations were made with a pH-stat of the Jacobsen and Léonis (1951) type with a Radiometer pH-meter coupled with an "Agla" micrometer syringe and recorder (Ole Dich, Copenhagen).

III. EXPERIMENTAL

All values refer to wool or oxidized wool of zero moisture content.

(a) Ion-exchange and Acid-uptake Properties of Oxidized Wool

Samples of normal and oxidized wools were washed in a large excess of 0.001N hydrochloric acid for 24 hr and then washed free of acid, dried, and equilibrated in air. The acid-uptake and ion-exchange properties were then studied in three ways:

- (1) Samples (1 g) were shaken (using a wrist-action shaker) for at least 30 min with 20 ml 1N potassium chloride solution containing either 1N or 0.05N hydrochloric acid. These conditions are more than adequate for the attainment of equilibrium (Alexander *et al.* 1951). 1-ml samples of solution were then back-titrated with standard 0.1N alkali (Alexander, Fox, and Hudson 1951). The experiments were carried out in replicate.

- (2) Samples (1 g) of oxidized wool were shaken successively (using a wrist-action shaker) for 15–30 min with 30-ml aliquots of 1N potassium chloride at pH 6.0 and the pH was measured at the completion of each shaking. Another sample (0.5 g) of the oxidized wool was packed in a column 1.0 cm in diameter and eluted successively with 100-ml portions of 1N potassium chloride (pH 5.8). The pH of the eluate was measured and the eluate then titrated to pH 5.8–6.0 with standard alkali.
- (3) Samples (0.5 g) of oxidized wool were titrated, with stirring, in 100 ml 1N potassium chloride to pH 5.7 with a pH-stat and the total amount of alkali consumed was determined.

(b) Equilibration of Oxidized Wool with Ammonium Chloride Solution

Oxidized wool (0.5 g) was successively equilibrated five times with 20 ml 1N ammonium chloride (pH 4.7) solution. Usually the mixture was left standing 2–16 hr before changing the aqueous medium. After the fifth equilibration the oxidized wool was carefully washed with water (5×20 ml) till free from chloride and ammonium ions (Nessler's reagent). The wool was then eluted with 20 ml 1N barium acetate solution at pH 6.5 and after 5 min the ammonium ions liberated into the supernatant were estimated after distillation from an alkaline solution into boric acid and titration with 0.02N acid.

(c) Equilibration of Oxidized Wool with Ammonium Phosphate Buffer

Oxidized wool (1 g) was equilibrated with 20 ml 0.28M ammonium phosphate buffer of pH 6.5 ($\text{NH}_4\text{H}_2\text{PO}_4$ 13.8 g and $(\text{NH}_4)_2\text{HPO}_4$ 10.6 g in 1l. of solution). After standing overnight the oxidized wool was swollen and some material had dissolved (cf. O'Donnell and Thompson 1959). After carefully washing with distilled water the filtrate gave a negative test for phosphate by the benzidine-molybdate reagent (Vogel 1945) and only a very slight positive test for ammonium ion by Nessler's reagent. (That phosphate was almost entirely absent was shown by the fact that tap water, containing only traces of phosphate (0.02 mg/l), gave a positive test.) During water-washing, the fibres swelled to a gelatinous material that could only be filtered off slowly on a Buchner funnel using Whatman No. 54 filter paper. A sample of oxidized wool which had previously been heated to 105°C for dry weight determination did not swell to the same extent. The residual oxidized fibre (a correction was made for absorbed water) was eluted with 10 ml 1N barium acetate solution (pH 6.5) and after 5 min the ammonium ions liberated into the supernatant were estimated as described in the previous section.

(d) Demonstration of the Formation of an Ammonium Salt by α -Keratose

A solution of α -keratose, prepared by extraction at pH 11 of performic acid-oxidized wool (O'Donnell and Thompson 1959), was dialysed overnight in a rocking dialyser against 0.1M ammonia solution. Aliquots (2 ml) of the solution of α -keratose were evaporated in a vacuum over sulphuric acid and caustic soda. Equal-sized aliquots were similarly evaporated for 3 hr and then 2 ml 0.2M potassium carbonate were added, followed by evaporation in a vacuum overnight to remove any bound ammonia. The nitrogen contents of the total dried residues were than determined by the Kjeldahl procedure (Chibnall, Rees, and Williams 1943).

IV. RESULTS AND DISCUSSION

(a) Ion Exchange and Acid Uptake of Wool Oxidized with Peracetic Acid

(i) *Washing of Oxidized Wool with Potassium Chloride Solution.*—Oxidized wool (5 g) on shaking for 2 hr with 200 ml water (pH 5.5) caused the pH to fall to 5.2; hence the washed oxidized wool contains virtually no free acid. However, on shaking 1-g samples with 30 ml 1N potassium chloride solution (adjusted to pH 6.0) the pH fell to 3.3 and after six successive shakings with fresh potassium chloride the pH was only 3.65. Two subsequent shakings with 200 ml 1N potassium chloride raised the pH to 4.0. It can be seen that it would require many shakings to raise the pH to the value for unbuffered potassium chloride, i.e. approximately 5.8. In an effort to equilibrate the oxidized wool to pH 5.8 with 1N potassium chloride solution it was washed in a column. By slowly passing 1300 ml 1N potassium chloride solution through the column (containing 0.5 g oxidized wool) the pH of the effluent slowly rose from 3.3, that of the last 100 ml being 4.6. The amount of alkali required to titrate the total 1300 ml of effluent to pH 5.8 was equivalent to 407 μ -equiv/g oxidized wool. It can be seen that it is impracticable to wash oxidized wool with unbuffered 1N potassium chloride until the pH of the potassium chloride is unchanged by passage through the wool.

Alexander, Fox, and Hudson (1951) state that they first equilibrated their oxidized wool samples (mainly partially oxidized wools) at pH 6.0 by successive shakings with potassium chloride solution before determining the acid uptake. No further details were given and it is not clear whether, in fact, a pH of 6.0 was reached.

(ii) *Uptake of Acid.*—With 1N hydrochloric acid in 1N potassium chloride it was found that normal wool took up 810 μ -equiv. of acid per g of wool, which is in good agreement with literature values (cf. Alexander and Hudson 1954). However, oxidized wool under the same conditions took up only 160 μ -equiv. of acid per g of original wool, allowing for the 3.5 per cent. increase in weight of wool on oxidation. These results which were obtained at pH 0.45 were intended to show the difference in acid uptake of normal and oxidized wool assuming any sulphonic acid groups in oxidized wool would not be ionized at these low pH values. It appears that oxidized wool has an even lower acid uptake than normal wool and, assuming that sulphonic acid groups are present, this could be due to the fact that:

- (1) An acid-rich fraction dissolves on oxidation of wool with aqueous peracetic acid. However, even if the 1 per cent. of dissolved material (Thompson and O'Donnell 1959) were pure cysteic acid it would not account for this discrepancy.
- (2) Some of the oxidized wool protein dissolves in the potassium chloride-hydrochloric acid solution. This factor cannot be a major one since nitrogen estimations showed that only 4 per cent. of the wool dissolved.
- (3) The sulphonic acid groups present in oxidized wool are still ionized almost completely at pH 0.45 and the carboxyl groups are practically unionized in water-washed wool at the beginning of the acid-uptake experiments.

The acid uptake of oxidized wool in 1N hydrochloric acid-1N potassium chloride was compared with that in 0.05N hydrochloric acid-1N potassium chloride. The acid uptake was small in both cases, being 66 μ -equiv/g for the 0.05N hydrochloric acid and 154 μ -equiv/g for the 1N hydrochloric acid.

With wool partially oxidized (1 hr) with peracetic acid (0.25N) Alexander, Fox, and Hudson (1951) found a maximum acid-combining capacity similar to that for untreated wool. Treatment with alkali gave an increase in acid-combining capacity. If, as discussed earlier, Alexander, Fox, and Hudson had, in fact, equilibrated their samples to pH 6.0 before titration with acid the carboxyl groups would have been ionized. They would then have back-titrated these ionized carboxyl groups and consumed acid equivalent to the alkali taken up during our equilibration (to pH 5.7) with the pH-stat. We are unable to explain their finding that partially oxidized wools on treatment with alkali had an acid-combining capacity in excess of that of untreated wool.

(iii) *Titration of Oxidized Wool with a pH-stat with Sodium Hydroxide.*—From the acid-uptake experiments and the fall in pH on washing wool with potassium chloride solution it must be concluded that factor (3) above is correct. Titration of the oxidized wool to pH 5.7 with the pH-stat showed that the wool took up 740 μ -equiv. alkali per g of oxidized wool over a period of 21 hr, less than 10 μ -equiv. being taken up over the last 6 hr. The wool which had been exhaustively washed on a column with potassium chloride when similarly titrated to pH 5.7 took up, over the course of 3 hr, an additional 236 μ -equiv. of alkali (per g oxidized wool) making a total alkali uptake of 643 (236+407) μ -equiv. (see Section IV(a)) alkali per g of oxidized wool. This latter value is not as accurate a value for the alkali consumption as that obtained by direct titration because of difficulties in the accurate titration of large volumes of the 1N potassium chloride from the column in which the oxidized wool was washed.

Since the uptake of hydrochloric acid by oxidized wool in the pH range 1.43-0.45 is only 66-154 μ -equiv. whereas the alkali consumed in titrating the oxidized wool to pH 5.7 is 740 μ -equiv/g, it is concluded that the carboxyl groups in oxidized wool are substantially unionized while the sulphonic acid groups are ionized.

It was further observed that on washing oxidized wool with water at pH 5.5 no protein dissolved but when 1N potassium chloride was present and the pH kept at pH 5.7 a substantial amount of wool (14 per cent.) dissolved. Ionization of the carboxyl groups, which is possible in the presence of a counterion (K^+), must be a prerequisite for solution of the oxidized wool in aqueous media.

(b) *Ion-exchange Characteristics of Oxidized Wool*

When oxidized wool was treated with ammonium chloride solution of pH 4.7 the pH fell within 2 min to pH 3.4 and only fell by a further 0.05 units overnight. Repeated treatment with fresh 1N ammonium chloride solution did not affect this behaviour which is similar to that reported for oxidized wool with solutions of potassium chloride. After five successive treatments the oxidized wool was washed with water until it was free from chloride ions and eluted with barium acetate

solution when it liberated its bound ammonium ions. Estimation of this displaced ammonia showed that only 79 μ -equiv/g of oxidized wool were present.

If $-\text{SO}_3\text{-H}^+$ groups capable of ion exchange were present with characteristics similar to those of a sulphonic acid type of cation-exchange resin (e.g. sulphonated polystyrene) ammonium ions equivalent to the $-\text{SO}_3\text{-H}^+$ groups should have been bound (731 μ -equiv/g of oxidized wool (Table 1) and more if sulphinic acids with a pK value of about 1-2 are present) as pointed out by Alexander, Fox, and Hudson

TABLE 1
ACIDIC AND BASIC GROUPS IN MERINO 64'S WOOL
Values in μ -equiv/g dry wool or oxidized wool

Group	Normal Wool		Wool Oxidized with Aqueous Peroxyacetic Acid
	Simmonds (1956)	Corfield and Robson (1955)	Corfield, Robson, and Skinner (1958)
Aspartic acid	476-537	511	511
Glutamic acid	1031	990	953
Cysteic acid	—	—	729 ^{722†} 742*† } 732
α -Carboxyl groups	10§	10§	(10)¶
Amide groups	745‡	786	1047 (720)¶
Total acidic groups	772-833	725	1483
Arginine	520	557	538
Histidine	53	74	63
Lysine	197	229	225
α -Amino groups	28	28	(28)¶
Total basic groups	798	888	854

* This value increases to 838 on further oxidation with performic acid (Thompson and O'Donnell 1959).

† Thompson and O'Donnell (1959).

‡ Leach and Parkhill (1956).

§ Bradbury (1958).

|| Thompson (1959).

¶ Values for unoxidized wool.

(1951). However, if the $-\text{SO}_3^-$ groups are internally compensated by the ionized basic groups in the oxidized wool they are not available for ion exchange and only the excess unionized carboxyl groups would be capable of exchanging ions. Under these conditions the binding of ammonium ions from an ammonium chloride solution by oxidized wool would be expected to be small since the weak carboxylic acid groups will not exhibit ion-exchange behaviour at low pH values.

The oxidized wool behaves like a carboxyl (weak acid) type of cation-exchange resin (e.g. "Amberlite IRC-50") the properties of which have been discussed by

Kunin and Barry (1949). Thus, in the hydrogen cycle the $-\text{COOH}$ groups are capable only of adsorbing cations present as the hydroxides, carbonates, or salts whose acids are not sufficiently strong to cause the pH to fall below 4–5 during the experiment. With salts of strong acids, as the pH falls to below 4 only hydrogen ions are held by the carboxyl groups due to the marked affinity of carboxyl groups for these ions. To lower the pH of 20 ml of 1N ammonium chloride solution from 4.7 to 3.5 requires only 6 μ -equiv. of hydrochloric acid. Thus, only a small proportion of the carboxyl groups in oxidized wool (Table 1) would bind ammonium ions from ammonium chloride solution before the pH fell to a value at which no uptake of ammonium ions by carboxyl groups would be expected. We can therefore account for the failure of Alexander, Fox, and Hudson (1951) to detect ion exchange with ammonium chloride or potassium chloride solutions. The 79 μ -equiv/g detected in our experiments represents the small amount of ammonium ion taken up before the pH fell to a value at which exchange ceased.

When an ammonium buffer of pH 6.5 was substituted for ammonium chloride solution the pH fell to 6.35 after standing overnight. On washing free of ammonium and phosphate ions the residual wool was eluted with barium acetate solution and ammonium ions equivalent to 476 and 532 (mean 504) μ -equiv/g of oxidized wool were liberated. When a carboxylic acid resin (1 ml wet "Amberlite IRC-50") in the acid form was substituted for oxidized wool in this experiment similar behaviour was observed. The pH fell to 6 in about 7 hr and after standing overnight with fresh buffer the pH was 6.35. The quantitative figures for bound ammonium ion in the exchange experiment with oxidized wool will be low for the following reasons. Firstly, if the time required for equilibration is similar to the 7 days required by carboxylic resins (Kunin and Barry 1949) the exchange cannot be continued for the necessary time since the oxidized wool is dissolving. Thus O'Donnell and Thompson (1959) found 34 per cent. of the nitrogen was extracted in 24 hr at pH 6.5 in the absence of salt. Secondly, being the "salt" of a weak acid ammonium ions will be lost by hydrolysis during washing of the ammonium "salt" to free it of excess buffer. However, it is clear that there is in oxidized wool a large number of carboxylic acid groups capable of ion exchange. Further, Hale and Reichenberg (1949) have shown that there is a spread of pK values in carboxylic ion-exchange resins and that the capacity for cations (other than hydrogen ion) increases with cation concentration and pH. The resin does not exhibit its full capacity below pH 8–9. With oxidized wool it was found that with a 10-fold increase in ammonium ion concentration (2.8M ammonium phosphate at pH 6.5 and equilibrating for 64 hr) the bound ammonium ions displaced by the barium ion increased to 693 μ -equiv/g oxidized wool. This value agrees well with the alkali-uptake value (740 μ -equiv/g). The increased recovery with the more concentrated buffer is probably due to the decreased extraction of wool proteins in the presence of salt (O'Donnell and Thompson 1959).

The results of both the acid-uptake and ion-exchange experiments therefore support the conclusions of Weston (1955) and Earland and Knight (1955) that oxidized wool contains $-\text{SO}_3^-$ groups but these are internally compensated by the basic groups in the wool. The pK of these sulphonie acid groups must be near zero

since they are still ionized at pH 0.45. The pK of the sulphonic acid groups in cysteic acid is 1.3 (Andrews and Schmidt 1927) but when involved in "salt-linkage" the pK values for acidic groups have been lower than normal (Lemin and Vickerstaff 1946).

In our previous work it was shown that 742 μ moles of cysteic acid are present per g of oxidized wool (Table 1) which increases to 838 on further oxidation with performic acid. The state of these 96 μ moles of partially oxidized cystine is not known but it could be in a partially or wholly acidic form, e.g. $-\text{SO}_2\text{H}$, so that the maximum possible number of "sulpho" acids in wool oxidized with peracetic acid is 838 μ moles/g of oxidized wool.

There is general agreement that the isoelectric point of wool is less than 7, so that at neutral pH the number of ionized negative groups exceeds the number of ionized positive groups. The amino acid composition of normal wool given in Table 1 compares the analytical values of Simmonds (1956) using the value for amide nitrogen of Leach and Parkhill (1956) with those of Corfield and Robson (1955). The former show a small excess of acidic residues particularly if a correction is made for the small proportion of histidine residues ionized at pH 7 (pK approx. 6) whereas the latter show an excess of basic residues. Hence the values of Simmonds combined with the amide values of Leach and Parkhill are likely to be the best at present available.

Treatment of wool with peracetic acid would not be expected to increase the content of amide groups; rather a decrease would be expected. The very high value of 1047 μ -equiv/g reported by Corfield, Robson, and Skinner (1958) may be due to increased ammonia liberation on treatment with acid in the presence of sulphonic acid groups (which catalyse hydrolysis reactions) in the oxidized fibre (Steinhardt and Fugitt 1942; Sanger 1949b). For this reason, and bearing in mind the arbitrary nature of the methods for amide estimation, we have chosen the value of 720 μ -equiv/g (the value for whole wool corrected for the increase in weight during oxidation).

From Table 1 the basic groups of oxidized wool total 854 μ -equiv/g, the free carboxyl groups 754 μ -equiv/g, and the cysteic acid groups 742 μ -equiv/g (838 μ -equiv/g if all possible "sulpho" acid groups are included).

Titration of oxidized wool with the pH-stat indicated the content of unionized carboxyl groups to be 740 μ -equiv/g and ionized carboxyl groups may contribute to the 154 μ -equiv/g of acid required to titrate the wool to pH 0.45. The value is therefore in good agreement with the value of 754 μ -equiv/g for free carboxyl groups quoted above. Hence in washed oxidized wool the 854 μ -equiv. of basic groups per g must be internally compensated by the 742 μ -equiv. of cysteic acid groups together with any sulpho acid groups—Cavallino *et al.* (1958) estimate a value of 2.16 for the pK of ethane sulphonic acid and Lavine (1936) a value of 2.1 for cystine sulphonic acid—and perhaps a small number of ionized carboxyl groups.

The calculations in this paper suffer from an inherent degree of uncertainty due to variation in the literature values for the amino acid composition of wool. Further work is obviously necessary to fix these values more accurately.

Earland and Knight (1955) claimed that whereas cysteic acid formed a monoammonium salt, α -keratose did not. We have confirmed their first finding and showed that treatment of the cysteic acid salt with potassium carbonate to remove ammonium ions causes the nitrogen content to revert to the value for cysteic acid. However, after dialysis of a solution of α -keratose to equilibrium with 0.1N ammonia and evaporation of excess ammonia in a vacuum the Kjeldahl titres were 10.04, 10.10, and 10.07 ml of 0.02N acid. After removal of ammonium ions with potassium carbonate the titres for equal quantities of the α -keratose were 9.54, 9.64, and 9.56 ml. The difference of 0.49 ± 0.03 (S.E.) in the mean values of 10.07 and 9.58 is significant at the 0.1 per cent. probability level. The α -keratose used in these experiments contained an excess of acid side-chains of about 490 μ -equiv/g (Table 2). It can be calculated that if an ammonium ion is bound at each of the

TABLE 2
ACIDIC AND BASIC GROUPS IN α -KERATOSE FROM PERACETIC ACID-OXIDIZED WOOL
Calculated from analytical results of Corfield, Robson, and Skinner (1958)

Acidic Group	Content (μ -equiv/g)	Basic Group	Content (μ -equiv/g)
Aspartic acid	714	Arginine	594
Glutamic acid	1245	Histidine	47
Cysteic acid	425, 606*	Lysine	263
Amide groups	1171	Total basic groups	904
Total acidic groups	1394†	Excess acidic groups	490†

* Value of O'Donnell and Thompson (1959) for α -keratose from performic acid-oxidized wool.

† This value will vary according to the amide N value (see text).

excess carboxyl groups the nitrogen content of 16 per cent. for α -keratose would be increased by 4.3–4.8 per cent. depending on the amide nitrogen content of the preparation. This corresponds closely with the 5.1 per cent. increase reported above.

In conclusion it should be pointed out that with continuous washing in distilled water a protein approaches an isoionic condition. If the protein contains a large excess of anionic side-chain groups which differ greatly in their pK values, then in washing free from acid those with the higher pK values will remain in the unionized condition as acid is removed by water and those with the lower pK values will ionize (or remain ionized) in amount equal to the ionized cationic groups in the protein. The data presented here are in complete accord with such a system.

V. ACKNOWLEDGMENTS

The authors are indebted to Dr. J. A. Maclaren for helpful discussions and to Mr. W. B. Hall for the statistical analysis.

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