tests based on variance alone—which excludes the *t* test, but allows the *z* test, Behrens's test, and others of the sort—are valid for a skew distribution provided there is no kurtosis. But it must be noted that these variances are to be taken about the, usually unknown, true or population mean; otherwise, the *z* test for skew populations is only a very good approximation for all but the smallest samples.

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[1] *Curr. Sci.*, 1940, **9**, 312.

[2] *Archæological Survey of India*, *Memoir* No. 59.

An Empirical Statistical Formula

FOR moderately asymmetrical distributions the empirical relation:

$$\text{Mean} - \text{Mode} = 3(\text{Mean} - \text{Median})$$

seems to hold good with a surprising degree of precision. In 1895, Karl Pearson proved the approximation for the Type III curve and in 1917, Doodson gave a proof for more general types of frequency distributions. But the simplest case of the triangular distribution defies the law in a remarkable manner not apparently noticed before, probably because of the artificiality of this case. It may be worth while to note the following facts, at least on account of their suggestiveness of other possible distributions where the law may be wholly denied:

(1) For a triangular frequency distribution,

$$4 \leq \frac{\text{Mean} - \text{Mode}}{\text{Mean} - \text{Median}} \leq 4 + 3\sqrt{2} \doteq 8\tfrac{1}{4}.$$

(2) The greatest value $4 + 3\sqrt{2}$ occurs for a finite linear distribution starting from 0, and also for an infinite triangular distribution.

(3) The least value 4 corresponds, paradoxically, to the isosceles triangle distribution, for which all the three measures of location coincide and the ratio therefore takes an indeterminate form with the limiting value 4.

(4) The ratio of the differences (mean, mode) and (mean, median) is a homogeneous fractional function of the ranges to the right and left of the mode.

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[1] *Philosophical Transactions*, 1895, **186 A**, Pt. I, 343.

[2] *Biometrika*, 1917, **11**, 425.

[3] *The Annals of Mathematical Statistics*, 1938, **9**, 176.

Mechanism of Swelling of Cellulose

WHILE studying the swelling of cellulose in aqueous solutions of neutral salts, $ZnCl_2$, $Ca(CNS)_2$, $Zn(CNS)_2$ and of mineral acids HCl, HNO_3, H_2SO_4 and H_3PO_4, following observations were made.[1] With both the salt and acid solutions, when solutions are dilute there is a preferential absorption of the solute. This is distinctly noticeable until the concentration of the solution reaches values that cause high swelling. Further, there is a distinct preferential absorption of the cation of the salt at low concentrations of salt solutions and also in the initial stages of swelling in high concentrations. Neale[2] who made an exhaustive study of swelling of cellulose in NaOH made similar observations and concluded that swelling is caused by the initial formation of a sodium salt of cellulose, which then ionises giving a diffusible hydrogen ion and a non-diffusible cellulose ion, the principle of the Donnan equilibrium being applicable to such a system. The acidic nature of the cellulose suggests the possibility of the formation of zinc and calcium salts of cellulose, although such a reaction is out of question between cellulose and mineral acids.

It is also observed that swelling in these solutions is accompanied by the formation of hydrocellulose. This has been confirmed by measuring the fluidity, copper number and the dye absorption of the original cellulose and of the swollen material.

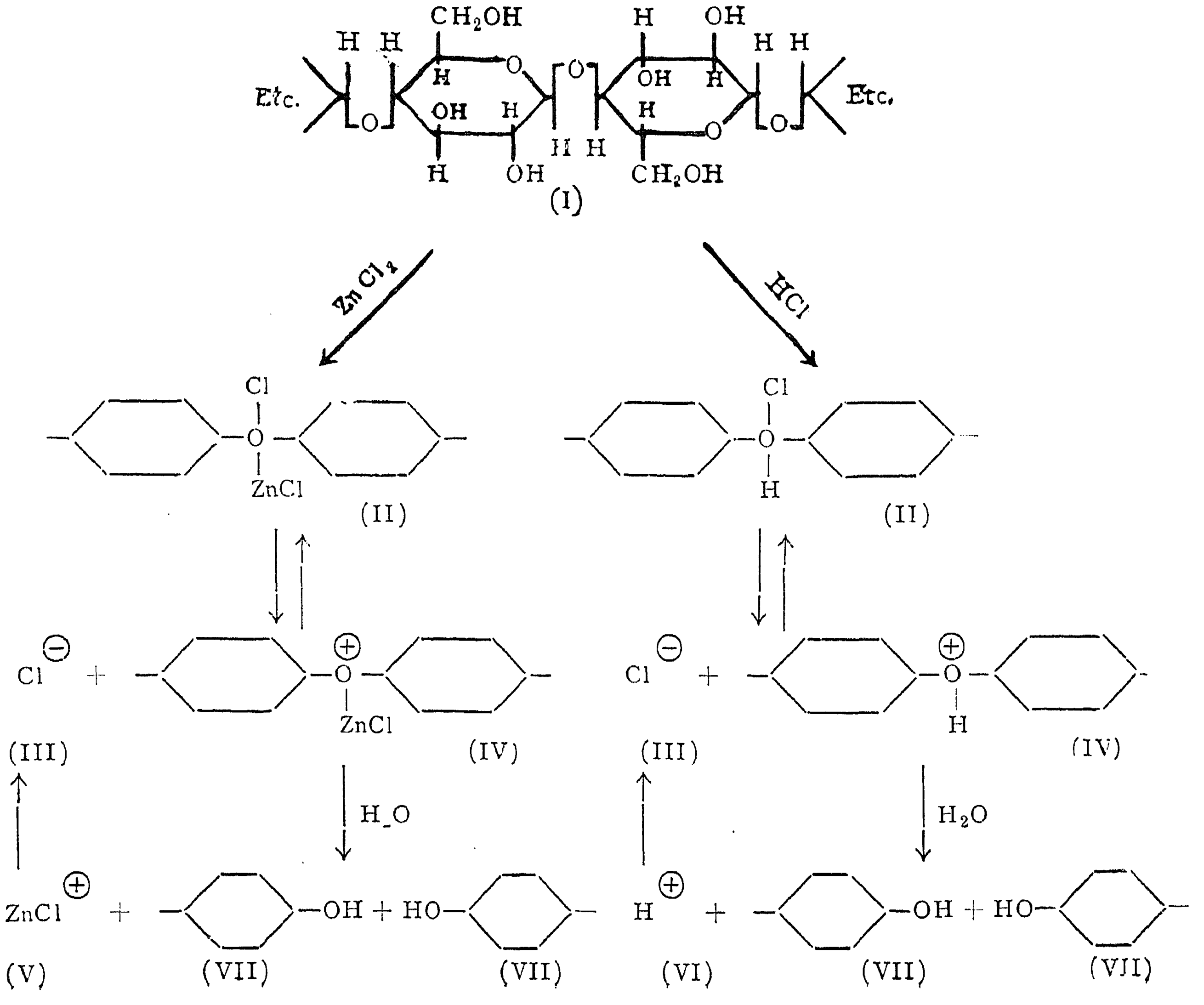
These results suggest the formation of some cellulose-salt or cellulose-acid complex of a type which results firstly in causing the swelling of the material and secondly in its degradation.

A consideration of the constitution of cellulose throws light on the nature of such complexes. Cellulose molecules consist of a large number of glucose units linked to each other through oxygen bridges. The oxygen atoms which form the glucoside linkage are divalent and by analogy with similar other organic compounds are, in all probability, capable of functioning as tetravalent atoms and forming oxonium salts with inorganic salts and acids. The work of Walker,[3] Knox and Richards[4] and of Baeyer and Villiger[5] indicates that the formation of oxonium salts with cellulose is distinctly possible. It is therefore suggested that the first stage in the swelling of cellulose is its formation of oxonium salts with salts and acids.

The following represents the changes which are suggested as taking place in the order in which they are presented.

The cellulose molecule (I) combines with the neutral salt or the acid to give the corresponding oxonium salt (II). It is further suggested that these oxonium salts in solution ionise and give rise to a diffusible anion (III) and to a non-diffusible 'oxonium cellulose cation' (IV) and thus create conditions which set up an osmotic flow of the external liquid into the gel phase in accordance with the principle of the Donnan equilibrium. This results in the swelling of cellulose.

The degradation of cellulose, which results in a shortening of its chain length and in the increase of the number of reducing end groups, can also be explained on the basis of the formation of oxonium salts and their subsequent ionisation. The non-diffusible 'cellulose oxonium cation' (IV) further reacts with a molecule of water and liberates the cations (V or VI) and simultaneously severs the glucoside link giving cellulose molecules (VII) of a shorter chain length. The cation from the salt or the acid bound to the cellulose is thus set free to combine with the anion formed at an earlier stage. This mechanism would also account for the hydrolysis of cellulose by acid or salt catalysis. The process of hydrolysis being a



slow one, it is obvious that "oxonium cellulose cation" will react with a water molecule only after an appreciable time. The formation of the oxonium compound, its ionisation, etc., would also account for the decrease in the 'ζ' potential with time in acid and salt solutions observed by Briggs.[6]

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[1] G. S. Kasbekar, *Ph.D. Thesis*, 1938, Manchester University.
[2] Neale, *J. Text. Inst.*, 1929, **20** T., 373–400.
[3] Walker, *J. Chem. Soc.*, 1904, **85**, 1105.
[4] Knox and Richards, *Ibid.*, 1919, **115**, 508.
[5] Bayer and Villiger, *Ber.*, 1901, **34**, 2679.
[6] Briggs, *J. Phys. Chem.*, 1928, **32**, 1646-62.

The Milk-Clotting Activity of Papain

It has already been shown that the gelatin splitting activity of papain is not impaired by oxidation of the fresh latex of papaya by hydrogen peroxide or alloxan; only the peptone hydrolysing activity is lost.[1] It has now been shown that the milk-clotting property of papain is also lost through oxidation with hydrogen peroxide or other oxidising agents.

Hydrogen peroxide-inactivated papain regains its milk-clotting property on reduction with cyanide or H_2S; as in the case of peptonase, this activity is inhibited by maleic acid (the inhibition being a time reaction), and the copper inhibition is annulled by cyanide. Iodoacetic acid irreversibly inactivates the enzyme. The press juice of pine apple and the latex of *Calatropis gigantea* behave exactly like papaya latex with respect to the milk-clotting activity. The minimum amount of iodoacetic acid required to arrest the milk-clotting properties of the papaya latex extract and pine apple press-juice of the same activity, is the same.

The changes in milk clotting activity brought about by treatment with different reagents are tabulated below.

Milk-clotting Activities of Papainases

(Figures indicate time in seconds required for clotting milk prepared from *klim*—5 c.c. of 10 per cent. in acetate buffer, pH 4·6 ; enzyme solution diluted with water or reagent : 0·5 c.c. ; temperature 40° C.)

Enzyme	Water extract	H_2O_2 treated	H_2O_2 treated + HCN	Copper 0·01 mg.	Maleic acid 5·8 mg.	Iodoacetic acid 0·01 mg.
Papain : latex extract 1 %	15	> 1 hr.	200	200	190	> 1 hr.
Pine apple press juice diluted with an equal volume of water	30	> 1 hr.	250	300	..	> 1 hr.
Calatropis gigantia latex extract 2 %	15	> 1 hr.	250	250	..	> 1 hr.

Balls and co-workers[2] observed that while preparing papain conditions which did not preclude oxidation always yielded preparations with low milk-clotting activity. This suggests that the milk-clotting activity is associated with a labile group and the observation reported above renders probable the assumption that this group is the SH group.

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July 5, 1940.

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[1] Ganapathy and Sastri, *Biochem. J.*, 1939, **33**, 1175.
[2] Balls and Lineweaver. *J. Biol. Chem.*, 1939, **130**, 669