

A Recalculation of Experiments on Mutarotation of D-Glucose and D-Mannose with an Interpretation of its Results

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A programme is described for calculating the equilibrium optical rotation of a solution from non-equilibrium data, referring to cases where only one reactant and one resultant are present. The calculations yield furthermore a well defined value for the equilibrium-fraction ω , (number of resultant moles)/(total number of moles).

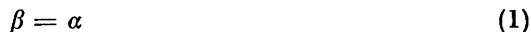
The programme is applied to measurements (Isbell and Pigman, Levene) on the reaction β -anomer \rightleftharpoons α -anomer for D-glucose and D-mannose. The result is that ω is very nearly 4/11 for glucose and 2/3 for mannose. The former fraction is independent of temperature and the same is probably true of the latter.

It is attempted to interpret the empirical results by means of mechanical and statistical-thermodynamical considerations of molecular models. The result is that the empirical values of the two fractions can and can only be interpreted in a natural way if it be assumed that the configurations of the two sugars at C₅, C₄, C₃, and C₂ are the current ones (Haworth, Hassel, Reeves) but that the HO-groups at C₁ are equatorial in the α -anomers and axial in the β -anomers (Blom).

We shall consider the case in which only the α - and β -anomeric pyranose forms of D-glucose and of D-mannose exist in measurable amounts in their aqueous solutions at equilibrium and during their transformation into each other; *cf.* Blom and Christiansen⁸.

Let us assume that we have at our disposal two series, α and β , of observations performed at the same temperature and with the same concentrations, beginning with pure α -anomer and pure β -anomer, respectively.

Let the stoichiometric equation be written



The velocity of (1) then is

$$v = k_+ C_\beta - k_- C_\alpha \quad (2)$$

while the equilibrium constant K becomes

$$K = k_+/k_- = (C_\alpha/C_\beta)_{\text{eq}} \quad (3)$$

Theory then yields

$$\begin{aligned} r_{\alpha} &= C + A \exp(-kt_{\alpha}) \\ r_{\beta} &= C - B \exp(-kt_{\beta}) \quad k \equiv k_{+} + k_{-} \end{aligned} \quad (4)$$

where r_{α} and r_{β} are rotations observed at times t_{α} and t_{β} respectively. If both series contain a sufficient number of observations it is always possible by interpolation to get sets of values for r_{α} and r_{β} at times $t_{\alpha} = t_{\beta} = t$. It is also possible to extrapolate to time $t_{\alpha} = t_{\beta} = \text{zero}$ with reasonable although not quite so good accuracy.

Evidently C means the equilibrium rotation which is a constant common to the two series. Omitting the subscripts at t in (4) we get by subtraction

$$r_{\alpha} - r_{\beta} = (A + B)\exp(-kt) \quad (5)$$

and by combination with the second (4)

$$C = r_{\beta} + \omega(r_{\alpha} - r_{\beta}); \quad \omega = B/(A + B) \quad (6)$$

where according to the theory

$$B/A = k_{+}/k_{-} = K = \omega/(1 - \omega) \quad (7)$$

Eqn. (6) shows that it is possible from the pairs of observations to determine a value of ω which causes C to be independent of time. The advantage of this method is, that it yields for each pair of observations one value of C and for the whole series a single value of ω , which must be so chosen, that any perceptible trend in the C -values disappears. The calculations will show that the determination of ω becomes rather accurate, the accuracy evidently depending on the accuracy of the experiments. The single C -values will scatter around some mean value which can easily be found, the same weight being ascribed to the different values.

From the existing measurements the author has primarily chosen those by Isbell and Pigman¹, because their experimental material seems to be homogeneous and their experiments very carefully performed.

D-Glucose. The author has found it convenient to assume ω to equal $(1 + x)/3$ for glucose and $2(1 + y)/3$ for mannose, where x and y are small fractions, the latter being probably zero inside the accuracy of the experiments. Table 1 shows the complete course of the calculation, starting from the interpolated values.

From Table 2 one may read the values of ω , the rotation $(r_{\alpha})_0$ of the pure β -anomer, the rotation $(r_{\beta})_0$ of the pure β -anomer and the equilibrium rotation C_{av} , where it must be remembered that $(r_{\alpha})_0$ and $(r_{\beta})_0$ are obtained by extrapolation. Of course the same quantities can be calculated from Isbell and Pigman's¹ formulae of the form (4). However, so far as ω is concerned, this procedure is ambiguous owing to the circumstance that the values chosen by I. and P. for the constant k differ a little at the same temperature. The same is true for the equilibrium rotation C . It is therefore not certain, that it has a meaning to take the mean of I. and P.'s values for C , but nevertheless we shall do so, adding these mean values in parentheses in Table 2. Concerning the initial values of r_{α} and r_{β} they must always be determined by some sort of extrapolation and it is difficult to tell, whether the simple linear extrapola-

Table 1. D-Glucose. Measurements by Isbell and Pigman¹ interpolated by J. A. Chr. Temp. 0.2°C. Concentration 3.9 g/100 ml, 4 dm tube. Rotations in saccharimeter degrees, °S. Time t in min. $\omega = 1.086/3 = 0.362$. C rotation in °S at equilibrium.

t	r_α	r_β	$r_\alpha - r_\beta$	$\omega(r_\alpha - r_\beta)$	C
0	50.79	8.37	42.42	15.36	23.73
5	50.54	8.49	42.05	15.23	23.72
10	50.34	8.68	41.66	15.09	23.77
20	49.83	8.87	40.96	14.83	23.70
40	48.96	9.39	39.57	14.33	23.72
60	48.14	9.85	38.29	13.86	23.71
90	46.96	10.56	36.40	13.17	23.73
120	45.85	11.19	34.66	12.55	23.74
180	43.72	12.41	31.31	11.34	23.75
240	41.83	13.49	28.34	10.27	23.76
300	40.07	14.52	25.55	9.25	23.77
400	37.56	15.93	21.63	7.83	23.76
550	34.46	17.65	16.81	6.08	23.73
1350	Interpolation uncertain			$C_{av} =$	23.74

Table 2. D-glucose. Concentration 3.9 g/100 ml, 4 dm tube. Temperatures in °C. Rotations in saccharimeter degrees, °S. The values in parentheses are taken from Isbell and Pigman¹.

Temp.	ω	$(r_\alpha)_0$	$(r_\beta)_0$	C
0.2	0.3620	50.79	8.37	23.74
0.2		(50.79)	(8.37)	(23.70)
20	0.3620	50.91	8.56	23.89
20		(50.95)	(8.54)	(23.96)

tion used in the present recalculation is better or worse than that implied in I. and P.'s tables. Here again the results of the latter authors have been included in Table 2 in parentheses.

To save space complete tables corresponding to Table 1 for glucose at 20°C and the corresponding tables for mannose have been omitted.

From Table 2 it appears, that ω is independent of temperature. This is corroborated by the findings of Nelson and Beegle² who extended their measurements from zero to 37°C. The same authors refer through secondary sources, unavailable to the present author, to a statement, probably about 100 years old, by Dubrunfaut and Mataqozeh to the effect, that the independency of temperature of the equilibrium ratio between the two forms of glucose extends to about 100°C. We shall therefore assume in the following that for D-glucose $K = \omega/(1-\omega)$ is to all intents and purposes independent of temperature.

It may be added, that the slight increase of ω from 0.3620 to 0.3636 would remove the last trace of a trend in the C values. This can be estimated from Table 1, and it is also true of the series of observations at 20°C. A similar further increase would reintroduce a trend but in the opposite direction, the first values becoming too high in relation to the last ones. We shall later see that it seems possible to give a reasonable interpretation of the value $4/11 = 0.3636$ for ω .

D-Mannose. Also in the case of mannose the measurements by Isbell and Pigman¹ seem to be among the most accurate ones. They are, however,

Table 3. D-Mannose. Concentration 4.0 g/100 ml, 4 dm tube. Temperature in °C. Rotations in saccharimeter degrees, °S. The values in parentheses are taken from Isbell and Pigman ¹.

Temp.	w	$(r_{\alpha})_0$	$(r_{\beta})_0$	C
0.3	2(1.026)/3	13.12	-7.60	6.55
0.3		(13.10)	(-7.62)	(6.65) ^a
20.1	2/3	13.70	-7.54 ^b	6.59
20.1		(13.70)	(-7.63)	(6.51) ^c

^a Mean of 6.63 and 6.66.

^b Possibly low because of uncertainty in extrapolation.

^c Mean of 6.63 and 6.38

peculiar in some respects. First of all the α -series have been obtained at 0.1°C and 20.0°C while the temperatures for the β -series were, respectively, 0.3 and 20.1°C.

The author has therefore referred the α -series to the latter two temperatures by the following procedure: From the values for the velocity-constant k given by Isbell and Pigman at the two temperatures it was calculated, that an increase in temperature of 0.2°C would increase the constant by 2.08 % and by the half of that value for an increase of 0.1°C. Therefore the times in the tables were reduced by these amounts, the rotations being left unchanged. After that the α, β tables at the two temperatures are comparable and were treated by interpolation as described under glucose.

The results of the recalculation are in Table 3.

Table 3 shows two peculiarities. In the first place w is essentially larger at about 0°C than at 20°C at which temperature it is exactly (inside the accuracy of the experiments) 2/3. The value 2/3 at 20°C was also obtained by the extremely careful measurements by Riiber and Minsaas³ which furthermore were discussed by N. A. Sørensen on pages 104 to 106 in his important book⁴ on the kinetics of mutarotation. Now, even if w were dependent on temperature it might happen that it became at 20°C exactly 2/3, but this would be a rather improbable incident. In the second place the rotation of the α -anomer at 0°C is essentially lower than that at 20°C. According to remarks by Nelson and Beegle² and by comparison with the data for glucose and for the β -anomer of mannose in this paper, this seems an improbable result. As we shall show, both peculiarities disappear if it be assumed that the α -specimen used by Isbell and Pigman for their experiment at 0°C has contained 2.5 % of the β -form.

In this connection a few words on the preparation of mannose may be of interest. According to Nelson and Sawyer⁵ β -mannose can be prepared in good yield from finely ground "ivory nuts", seeds from the tagua palm (*Phytelephas macrocarpa*) by hydrolysis with 75 % sulfuric acid. The α -anomer was first prepared by Levene⁶, who in a later paper⁷ revised and improved his method and his mutarotation experiments. It is thus evident that it is much easier to get the β -form free from the α -form than *vice versa*.

Note added in proof. Riiber and Minsaas³ recommend a different procedure. They show that, if a laboratory has become contaminated by crystal nuclei of either of the two forms, the preparation must be performed under "aseptic" conditions to get pure products.

Now if we start an α,β -pair of experiments with the pure β -form but with an α -specimen containing the fraction β of the β -anomer it can be shown that, by the method described above, we shall arrive at an apparent value of ω , $\omega_{\text{app}} = \omega/(1-\beta)$. Now from $1/0.975 = 1.0256$ we conclude that the α -specimen at 0.3°C may have contained very nearly 2.5% of the β -form. Furthermore, if we calculate $(r_\alpha)_o = x$ from C at 0.3°C (which of course is independent of the fraction β) under the assumption that $\omega = 2/3$, we get from Table 3

$$2x/3 - 7.60/3 = 6.55$$

or at 0.3°C $(r_\alpha)_o = 13.63^\circ\text{S}$ which is not more different from the value 13.70 at 20°C than could be expected.

Recalculation of the apparent rotation of the α -form then yields $(r_\alpha)_{\text{app}} = 13.10^\circ\text{S}$, which is not significantly different from the 13.12°S at 0.3°C in Table 3.

However, although the results of Isbell and Pigman may be consistent with the assumption, that $\omega = 2/3$ independently of temperature, more direct evidence would seem to be needed. To this end the author has recalculated the experiments by Levene in his second paper¹, where he has taken the utmost care to get a pure α -specimen. The results are in Table 4.

Although Levene's data are, relatively, a little less accurate than Isbell and Pigman's, it appears from Table 4 that $2/3$ is a better choice for ω than $0.684 = 2(1.026)/3$. For lack of further information we therefore adopt the value $2/3$ for ω at all temperatures.

The result of the recalculation is then that for D-glucose the fraction ω , that fraction which is present at equilibrium in aqueous solution as α -glucose, is very nearly $4/11$ at all available temperatures and that the corresponding β -fraction therefore is $7/11$.

For D-mannose the α -fraction is $2/3$ at 20°C . It seems probable, experimentally, that the same is true at 0°C , but a strict experimental proof would require further experiments. Evidently then the equilibrium fraction of β -mannose is $1/3$.

INTERPRETATION OF THE RESULTS FOR D-GLUCOSE AND D-MANNOSE

We shall treat the equilibrium between E- and A-molecules of the two sugars. For both the E-form is the one in which the HO at C_1 is located equatorially and the A-form the one in which it is located axially (and "southwards")⁸. We assume the solution to behave ideally, that is, we assume that interactions between solute molecules can be neglected. The solvent is supposed to be water, which by exchange with the solutes cannot produce perceptible changes. We write the reaction as

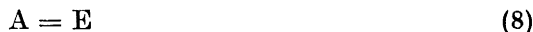


Table 4. Experiments by P. A. Levene⁷. Temperature 1°C . Concentration 10%, 2 dm tube. Rotations in angular degrees. Time in min.

Time	0	19	40	60	90
$C(\omega = 0.684)$	2.92	2.92	2.89	2.85	2.87
$C(\omega = 2/3)$	2.77	2.78	2.77	2.75	2.79
$C_{\text{av}}(\omega = 2/3)$	2.77				

Therefore the equilibrium constant K becomes

$$K = (C_E/C_A)_{\text{eq}} \quad (9)$$

Glucose. It was shown by Blom and the author⁸ that for glucose K must approximate 1/2. In the following we shall therefore consider $2K$ instead of K .

From thermodynamics it is known that

$$RT \ln(2K) = \mu_A - \mu_E \quad (10)$$

where the μ 's are chemical potentials of the pure substances at unit (or equal) concentrations. The difference can be written

$$\mu_A - \mu_E = H_A - H_E + T(S_E - S_A) \quad (11)$$

If the temperature interval is not too large the differences in the right side of (11) can be considered as being constants, representing the two differences at some standard temperature T_0 chosen inside the temperature-interval covered by the measurements. This is so because of the fact that at the temperature $T = T_0$ both the function O and its first differential quotient dO/dT disappear, O being defined as

$$O \equiv \int_{T_0}^T C_p dT - T \int_{T_0}^T (C_p/T) dT \quad (12)$$

The experiments show that K is independent of temperature, which means that $H_A = H_E$. The dimensionless difference $(S_E - S_A)/R$ is then also a constant which we denote by $\ln \sigma$. Assuming as we did, that the numerical value of ω is 4/11 we get

$$2K = 8/7 = \sigma \quad (13)$$

The question then is, how are we to interpret this empirical result? To answer the question we use statistical thermodynamics although with some simplifications.

It is known⁹ that an assembly of molecules of the same kind at unit concentration has a chemical potential μ given by

$$-\mu/RT = -H/RT + \Sigma \ln({}^i Q) \quad (14)$$

where ${}^i Q$ is the partition function (german: Zustandssumme) relating to the i 'th type of motion of the molecule. (14) implies that the different types of motion (*e.g.* translations, rotations, vibrations) can, with sufficient approximation, be considered as non-interfering with each other. H and only this is in principle independent of temperature. Applying (14) to (10) we get

$$\ln(2K) = (H_A - H_E)/RT + \Sigma \ln({}^i Q_E / {}^i Q_A) \quad (15)$$

The constants H include in the first place the potential energy of the molecule. The E- and A-molecules being alike except for the configuration at C_1 we need only consider the "bond energies" of H and OH at this carbon atom. The bond energies are defined as the work necessary to remove the atom or the group from its equilibrium place in the molecule into infinity. It is natural to assume that these bond energies are very nearly independent of the locations (*e-* or *a-*) of the atom or the group in question, provided that only forces

between them and C_1 come into play. If for example there were an OH at C_3 located axially southwards it might interact strongly with an OH at C_1 also located axially southwards. In such a case we may expect the bond energy of the OH at C_1 to be significantly larger in the A-form of the molecule than in the E-form. Such cases will be treated by J. Blom, who has drawn the authors attention to this possibility. However, neither in D-glucose nor in D-mannose can a complication of this kind arise. The constants H_E and H_A may beside the bond energy also contain contributions of vibrational origin, namely so-called zero-point energies. These arise from the circumstance that, according to quantum-mechanics, a harmonic vibrator is never at rest not even at 0°K. It has at least the energy $h\nu/2$ where ν is the frequency of the vibrator calculated according to classical mechanics. These vibrations must be considered as vibrations of different characteristic frequencies of vibration of the entire molecule superimposed on each other and cannot therefore, strictly speaking, be ascribed to single bonds. But some of them may approximately be ascribed to vibrations at bonds which are either unique or are repeated in several places in the molecule. An example of the unique kind is the bond between C_5 and C_6 . This bond may give rise both to longitudinal and to transversal vibrations, these expressions being understood as relating to the direction of the "valency" between the two carbon atoms. The restoring forces necessary to produce such vibrations must arise mainly from the electron-cloud surrounding the bond in question and must therefore be nearly equal for the E- and the A-form of the molecule. Thus the corresponding energies cancel in the difference $H_A - H_E$. An example of the "repeated" kind are the bonds $H-C_5$, $H-C_4$, $H-C_3$ and $H-C_2$. These are common to the E- and A-form and their energy will cancel in the difference above. Assuming as we did before, that the restoring forces at a bond are mainly determined by the electron-cloud surrounding the bond in question we may with some confidence assume that the frequencies (longitudinal and transversal) of the C_1-H bond as well are the same in the E- and the A-form. The situation is similar so far as longitudinal vibrations of the four bonds $HO-C_4$, $HO-C_3$, $HO-C_2$ and $HO-C_1$ are concerned. The question of transversal vibrations at the same four bonds will be treated below, but it may be stated at once that the empirically found equality between H_A and H_E shows that the energy of the collective transversal vibrations of the mentioned four vectors $HO-C$ actually is the same for the two isomers.

Next we discuss the partition-functions Q . The first one may be taken as the one belonging to the translational movements of the molecule as a whole, tQ . We get one such Q for each of the three directions in space. Each of them is given by the familiar expression

$${}^tQ = \int_{-\infty}^{+\infty} \exp(-p^2/2mkT) dp = \sqrt{2\pi mkT} \quad (16)$$

The three tQ 's are therefore equal for the two forms and their ratio disappears from eqn. (15). It is much more complicated to calculate the three possible ${}^{\text{rot}}Q$'s corresponding to the three possible rotations of the entire molecule, but owing to the fact that the three fundamental moments of inertia of the

molecule are large, it can be estimated, that also the three ${}^{\text{rot}}Q$'s must be equal for the two isomers (E and A). Therefore also the corresponding ratios disappear from (15). What remains on the right side of (15) are then different vibrational partition functions ${}^{\text{vib}}Q$'s each depending on its own characteristic frequency, ν . It is known from textbooks that, omitting the superscript

$$Q(\nu) = (2\sinh(x/2))^{-1}; \quad x = Lh\nu/RT \quad (17)$$

where L is Avogadro's number.

There are two limiting cases to consider.

$$(a) \quad Lh\nu \gg RT: \quad 2Q(\nu) = \exp(-x/2) \quad (18)$$

$$(b) \quad Lh\nu < RT: \quad 2Q(\nu) = (x/2)^{-1} = 2RT/Lh\nu \quad (19)$$

For most of the vibrators considered conditions (a) will obtain because the restoring forces are large. This is probably true for all longitudinal vibrations and also for transversal vibrations of H-atoms. It may not be true for transversal vibrations of the vector C_5-C_6 but this is common to the two isomers and the corresponding Q -ratio therefore disappears from (15). In cases where (a) obtains the corresponding $\ln Q$'s evidently appear as contributions to H , the case we have already discussed.

Concerning (b) we note, that even if $Lh\nu = RT$ the approximation (19) is not a bad one, it being only about 4 % larger than the true one. What remains to discuss are the Q 's corresponding to transversal vibrations of the four HO—C vectors. So far as restoring forces due to the electron-cloud around the vector in question are concerned, these are expected to be nearly equal for the two isomers and the corresponding Q -ratios will therefore disappear from (15). But apart from this kind of vibrations there must be one, in which the restoring force is due to interaction between neighbouring equatorial HO-groups. These restoring forces must be rather weak so that we expect condition (b) to obtain. Of neighbouring equatorial HO-groups there are four in the E-anomer (of glucose). In the A-anomer there are only three equatorial C—OH vectors and one whose "valency" is parallel to the axis of the molecule. In both cases they will be strongly coupled to each other and form a collectively vibrating system with one characteristic frequency for each anomer, denoted by ν_E and ν_A , respectively. In this case there must be phase relations between the vibrations of the single vectors, most probably so that two neighbouring ones are always in counter phase.

The approximation (19) then yields, for this Q -pair

$$Q_E/Q_A = \nu_A/\nu_E \quad (20)$$

The energies U_A and U_E of the two collectives must be equal (U) because of the independence of $\ln(2K)$ of temperature, but the energy of each vibrator in the collective and therefore the frequency of vibration of the collective may be different for the two anomers. For the E-anomer U will be divided equally between the four equally situated C—OH vibrators. According to classical mechanics the vibrational energy of a harmonic vibrator is on the average half potential and half kinetical, corresponding to the two square terms in its energy expression, its Hamiltonian. Thus on each square term we shall have the energy $U/8$ and for one vibrator $2U/8$. Therefore $\nu_E = 2U/8h$.

In the A-anomer the situation is different so far as the HO—C₁ vector is concerned. The transversal restoring force of the kind considered can only come from the triplet of vectors at C₄, C₃ and C₂. The restoring force on HO—C₁ from this triplet must be parallel to the axis of the molecule and to the axial vector at C₁. It has therefore no component transversal to the HO—C₁ vector. The corresponding square term in the Hamiltonian will thus be missing, and only its kinetical square term will remain. The energy on each square term will therefore in the A-case be $U/7$ which yields $\nu_A = 2U/7h$. As all other Q -ratios equal unity we get from (15) and (20)

$$2K = 8/7 \quad (21)$$

which is the result found empirically for glucose, where $(C_\alpha/C_\beta)_{\text{eq}} = K = 4/7$.

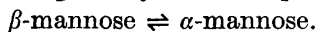
D-Mannose. In mannose we have at C₄, C₃ a doublet of equatorial C—OH vectors and at C₂ a singlet pointing axially northwards. In the A-anomer the singlet at C₁ points axially southwards. Using the same argument as we did in the case of A-glucose we see that two square terms are missing, one from each of the vectors at C₂ and C₁. In A-mannose we therefore have only $2 \times 2 + 2 = 6$ square terms in the Hamiltonian. In E-mannose the singlet at C₁ is equatorial but rather distant from the C₄, C₃ doublet, the angular distance between the middle of the latter and the singlet at C₁ being 150°. As now the two vectors in the doublet most probably vibrate in counter-phase the resulting transversal effect on the equatorial C₁-singlet will disappear and the restoring force on this vector becomes zero also in E-mannose. Thus we get again 6 square terms in the Hamiltonian and consequently

$$Q_E/Q_A = \nu_A/\nu_E = 1 \quad (22)$$

Remembering that the statistical factor for mannose is 2 and reasoning as we did in the case of glucose we find

$$K/2 = 1 \quad (23)$$

which is the result found empirically for the equilibrium



The empirical results for glucose and mannose can thus be interpreted in a manner which seems natural, but only if the current assignment of E- and A-forms to the α - and β -anomers be reversed.

REFERENCES

1. Isbell, H. S. and Pigman, W. W. *J. Research, Natl. Bur. Stand.* **18** (1939) 141, tables p. 179, 180.
2. Nelson, J. M. and Beegle, F. M. *J. Am. Chem. Soc.* **41** (1919) 559.
3. Riiber, C. N. and Minsaas, J. *Ber* **60** (1927) 2402.
4. Sørensen, N. A. *Kgl. Norske Videnskab. Selskab. Skrifter*, Trondheim (1937) Nr. 2.
5. Hudson, C. S. and Sawyer, H. L. *J. Am. Chem. Soc.* **39** (1917) 470.
6. Levene, P. A. *J. Biol. Chem.* **57** (1923) 329.
7. Levene, P. A. *J. Biol. Chem.* **59** (1924) 129.
8. Blom, J. and Christiansen, J. A. *Acta Chem. Scand.* **16** (1962) 1519.
9. As examples of text-books the following may be mentioned: Fowler, R. H. *Statistical Mechanics*, Cambridge University Press 1929; Fowler, R. H. and Guggenheim, E. A. *Statistical Thermodynamics*, Cambridge University Press 1939; Guggenheim, E. A. *Thermodynamics*, North Holland Publishing Company, Amsterdam 1949 and later editions.

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