RANGE OF ASCORBIC ACID AND PIP CONTENTS IN HIPS OF *Rosa canina*.

Weight of pips (per cent)	No. of observations	Mean vitamin C (mgm./100 gm.)	Range of vitamin C (mgm./100 gm.)
20–24	1	187	187
25–29	2	275	100–450
30–34	13	546	205–1055
35–39	28	501	115–850
40–44	23	508	164–1010
45–49	8	509	370–735
50–54	2	498	385–610
55–59	2	172	120–224
Total	79		

Species means: Vitamin C content, 493. Percentage of pips, 39.

limited but generally unfavourable. Thus hips of a bush of *R. canina* var. *dumalis* assayed in 1941 had 44 per cent pips and 164 mgm./100 gm. vitamin C; but in 1942, 29 per cent pips and only 100 mgm./100 gm. Corresponding figures for a bush of *R. mollis* were 1941: 24, 1,490; 1942: 15, 1,653; 1943: 23, 1,650; and for a bush of *R. dumetorum*, 1941: 51, 920; 1942: 32, 935; 1943: 33, 960. More numerous data published earlier by Schröderheim[3] on *R. pendulina* (Tables 9–12) and *R. rugosa* (Tables 33–34) appear to be no more conclusive.

It is noteworthy that of the three 'cases' cited by Gustafsson and Schröderheim, the correlation is lowest and least significant for the single species *R. rubiginosa*. The correlation is a little higher and significant for the mixture (Case 1) consisting mainly of *R. canina* and *R. Afzeliana*, and the only high correlation is for a heterogeneous mixture (Case 2). Probably this progression is due to an increased mingling of species having high vitamin and low pip content with others possessing low vitamin and high pip content. Among British roses the specific differences tend in these directions and appear to be due to hereditary differences in vitamin production coupled with differences in the proportions of the receptacle, and are independent of fertility. The diversity of vitamin content and fertility between the reciprocal hybrids of *R. canina* and *R. rubiginosa*, observed by Gustafsson and Schröderheim, can be regarded as another example of a kind of variation not uncommon among hybrids and genetical in origin.

RONALD MELVILLE.

Royal Botanic Gardens,
Kew.

[1] Pyke, M., and Melville, R., *Biochem. J.*, **36**, 336 (1942).
[2] Gustafsson, A., and Schröderheim, J., NATURE, **153**, 196 (1944).
[3] Schröderheim, J., *Kungl. Fys. Sällsk. Handl.*, **52**, No. 9 (Lund, 1941).

Preparation of Retinene *in Vitro*

RETINENE, obtained by Wald from dark-adapted retinas, is defined as the chromogen responsible for a maximum at 664 mμ in the absorption spectrum of the blue solution obtained by mixing retinal extracts with antimony trichloride in chloroform. Such extracts show (in chloroform) an ultra-violet absorption band with $\lambda_{max.}$385 mμ. It has recently been suggested[1] that the chromogen is vitamin A aldehyde, and that it can be obtained by direct oxidation of *vitamin A alcohol*.

The following procedure yields small quantities of retinene in a state approaching purity. 2 gm. of distilled vitamin A alcohol ($E^{1\%}_{1cm.}$ 325 mμ 800, *c.* 46 per cent vitamin A) is dissolved in 40°–60° petrol ether (50 c.c.) and the solution poured into 5 per cent sulphuric acid (400 c.c.) at 25° C. *N*/10 potassium permanganate (600 c.c.) at 25° C. is then run in rapidly with vigorous shaking for a few minutes. The mixture is allowed to settle in a separating funnel and the aqueous layer discarded. The petrol ether layer is washed twice with water and dried over sodium sulphate. It is then poured on to a column of alumina (Brockmann) and the chromatogram developed with petrol ether. The solute is gradually washed through the column and the fractions are tested separately (1 or 2 drops with 4 c.c. antimony trichloride reagent). If each successive 10 c.c. portion of eluate is regarded as a 'fraction', the results can be summarized as in the accompanying table.

Fractions containing the 664 mμ chromogen only show

	664 mμ	>3200
$E^{1\%}_{1cm.}$	361 mμ	1420 (petrol)
	385 mμ	1570 (chloroform)

while the best fractions for the 560 mμ chromogen show

$$E^{1\%}_{1cm.} \quad 560\ m\mu/355\ m\mu = 2:1.$$

For both substances, the molecular extinction coefficient for the ultra-violet maximum approaches that of vitamin A. The 664 mμ chromogen is undoubtedly retinene. It is by no means an unstable substance. In cyclohexane, the ultra-violet maximum falls near 368 mμ and in petrol nearer 361 mμ. The 560 mμ chromogen (the nature of which cannot yet be discussed) is also a stable substance exhibiting a similar displacement of the ultra-violet maximum from 355 mμ in chloroform to 342 mμ in petrol. Hunter and Hawkins[2] in recording the preparation of vitamin A aldehyde by the Oppenauer method, give $\lambda_{max.}$657 mμ in the colour test and two maxima (368 and 350 mμ) for the ultra-violet absorption in cyclohexane. Wald at first gave 655 mμ for the colour test maximum, but later corrected it to 664 mμ. His ultra-violet maximum at 385 mμ refers only to chloroform solutions. The large solvent displacement, 368–385 mμ, which we have observed reconciles the data and makes it clear that retinene and vitamin A aldehyde are spectroscopically indistinguishable. We have not so far found the 664 mμ and 560 mμ chromogens to be

Fractions	Approx. conc. (per cent)	$\lambda_{max.}$ in $SbCl_3$ colour test: 620, 610, 583, 664, 560, 490 mμ	$\lambda_{max.}$ ultra-violet absorp. in chloroform, 325, 355, 385, 332, 300 mμ; in petrol, 325, 342, 361, 321, 287 mμ
1–10		+	+
11–13		+ ++	+ ++
24–26		++	++
27–41	0·06	+ +++	+++ +
42–50	0·02	++ +	+ ++
51–57	0·018	+++	+++
58–66	0·025	+	+
67–70	0·045	+	+ +

interconvertible, and the best fractions of the former show one ultra-violet maximum only.

The elegance and accuracy of Wald's work on retinal extracts makes us hesitate to suggest that the term *retinene* is inappropriate. Unfortunately, it suggests a retinal carotenoid. *Axerophthal*, following Karrer's terminology, is not very happy in this context. Perhaps *retinaldehyde* is more appropriate than retin*al*.

A fuller account of this work will be published elsewhere. We express our thanks to the Medical Research Council for financial assistance.

R. A. MORTON.
T. W. GOODWIN.

The University,
Liverpool, 3.
Feb. 25.

[1] Morton, NATURE, 153, 69 (1944).
[2] Hunter and Hawkins, NATURE, 153, 194 (1944).

Molecular Weight of Egg Albumin

THE molecular weight of egg albumin quoted in all but the most recent literature is 35,000–36,000, a value based on the early osmotic pressure measurements of Sørensen[1], the ultracentrifuge measurements of Svedberg and Nicols[2], Nicols[3], and Sjögren and Svedberg[4], as well as the diffusion data of McBain[5]. More recently, however, there has been general agreement that this value is too low; the sedimentation constant and diffusion data quoted by Svedberg and Pedersen[6], for example, as well as the osmotic pressure data summarized in Table 1, indicating a value of 43,000–46,000.

TABLE 1.

Author	Solvent used	Result
Sørensen	Water	34,000[1]
Adair (recalculated Sørensen's data)	Ammonium sulphate	43,000[7]
Marrack and Hewitt	Sodium acetate and sodium chloride	43,000[8]
Taylor, Adair and Adair	Sodium acetate	46,000[9]
Bull	Sodium acetate	45,160[10]

I have had occasion, while rehearsing the procedure to be applied to certain other proteins, to make osmotic pressure measurements with solutions of egg albumin. This protein was prepared by Prof. R. K. Cannan using the method of Kekwick and Cannan[11], and the amount in solution was estimated by the micro-Kjeldahl procedure and nitrogen figures of Chibnall *et al.*[12]. McIlwain's phosphate-citric acid buffer at *p*H 4·65 was used as solvent.

The osmometer used was of the type described by Adair[13], but the collodion membranes contained about 3 c.c. instead of 20 c.c. of protein solution. Pressures were calculated from the height of the column of solution of known density, corrected for the rise due to capillarity.

TABLE 2.

C_v	Pressure	Molecular weight
1·00	3·92	43,400
1·86	6·71	47,200
4·00	14·4	47,300
4·23	16·7	43,100
5·84	21·1	47,200
5·95	22·0	46,000
6·26	22·2	48,100
6·88	26·1	44,900
8·30	30·6	46,200
8·84	32·5	46,300
	Average	45,970

The computed values for the molecular weight are given in Table 2, where the pressures are recorded in millimetres of mercury at 0° C. It was found that over the range investigated the osmotic pressure was directly proportional to the protein concentration C_v, expressed in gm. per 100 c.c. solvent. The calculated results for the molecular weight, which are in agreement with those recorded in Table 1, though obtained under different conditions, show that the value is almost certainly 45,000 ± 2,000.

HERBERT GUTFREUND.

Biochemical Laboratory,
Cambridge.

[1] Sørensen, *C. R. Trav. Lab. Carlsberg*, **12**, 122 (1917).
[2] Svedberg and Nicols, *J. Amer. Chem. Soc.*, **48**, 3081 (1926).
[3] Nicols, *J. Amer. Chem. Soc.*, **52**, 5176 (1930).
[4] Sjögren and Svedberg, *J. Amer. Chem. Soc.*, **52**, 5187 (1930).
[5] McBain, Dawson and Barker, *J. Amer. Chem. Soc.*, **51**, 1021 (1934).
[6] Svedberg and Pedersen, "The Ultracentrifuge" (Oxford University Press, 1940), 382.
[7] Adair, *J. Amer. Chem. Soc.*, **49**, 2524 (1927).
[8] Marrack and Hewitt, *Biochem. J.*, **23**, 1079 (1929).
[9] Taylor, Adair and Adair, *J. Hygiene*, **32**, 340 (1932).
[10] Bull, *J. Biol. Chem.*, **137**, 143 (1941).
[11] Kekwick and Cannan, *Biochem. J.*, **30**, 232 (1936).
[12] Chibnall, Rees and Williams, *Biochem. J.*, **37**, 354 (1943).
[13] Adair, *Proc. Roy. Soc.*, A, **108**, 627 (1925).

Number of Configurations of Molecules occupying Several Sites

IN a recent communication[1], I gave a formula for $g(N_i)$ the total number of configurations of a mixture of molecules of several types, N_i denoting the number of type i. I have meanwhile obtained the more general formula for $g(N_i,X_{ij})$, the number of configurations of the molecules in which the number of pairs of sites occupied in alternate ways is specified.

Let the number of sites which are neighbours of one site be z; let each molecule of type i occupy r_i sites; let the number of sites which are neighbours of a molecule of type i be q_iz. The r_i's and q_i's are related by $z(r_i - q_i) = 2(r_i - 1)$. Let the number of alternative configurations of a molecule of type i be ρ_i when a site has been chosen for one of its elements. Let the number of pairs of neighbouring sites, one occupied by a molecule of type i the other by a molecule of different type j, be denoted by zX_{ij}.

The number $g(N_i, X_{ij})$ of distinguishable configurations of given N_i and X_{ij} is given by

$$\log g(N_i,X_{ij}) = \Sigma_i N_i \log \rho_i - \Sigma_i \log N_i ! + z\Sigma_i \log (q_iN_i) ! - (\tfrac{1}{2}z - 1) \log (\Sigma_i r_iN_i) ! - \tfrac{1}{2}z \Sigma_i \log (q_iN_i - \Sigma'_k X_{ik}) ! - z\Sigma'_{ij} \log X_{ij} !,$$

where Σ' denotes summation over all types except i. This includes as special case a formula due to Chang[2] for a mixture of two types of molecule, of which one type occupies two sites, the other a single site.

The above formula can be used to derive the thermodynamic properties of mixtures with non-zero energies of mixing, whereas my previous formula was sufficient for mixtures with zero energies of mixing. In particular, I find that T_c, the temperature of critical mixing of a binary mixture of molecules A and B, is related to w, the intermolecular energy per pair of sites one occupied by part of an A the other by part of a B molecule, by the formula

$$e^{2w/kT_c} = \tfrac{1}{2}\{1 + ab + \sqrt{(a^2 - 1)(b^2 - 1)}\},$$

where $a = zq_A/(zq_A - 2)$ and $b = zq_B/(zq_B - 2)$.