THE MECHANICAL PROPERTIES OF THE THICK WHITE OF THE HEN'S EGG

J. BROOKS AND H. P. HALE

Low Temperature Station for Research in Biochemistry and Biophysics, University of Cambridge and Department of Scientific and Industrial Research, Cambridge (Great Britain)

(Received May 13th, 1958)

SUMMARY

1. The thick white fraction of the egg consists of a gel in which a transparent phase is separated by a series of parallel bands of microscopic fibres. Its mechanical properties have been studied by measuring in a magnetic field the displacement of a minute nickel sphere inserted into the gel.

2. The values of the rigidity in arbitrary units obtained in this way were converted indirectly into absolute units by means of parallel measurements on gelatin gels using, on the one hand, the magnetic particle method, and on the other, a method that measured rigidity in c.g.s. units.

3. For stresses of short duration, the displacement of the particle was elastic in character in both the transparent phase of the thick white and in the bands. The thick white, therefore, is a weak gel interpenetrated by a system of microscopic, elastic fibres and not, as is usually believed, merely an entanglement network.

4. The rigidities of the two types of structure in the thick white were different but both of them were related quantitatively to the proportion of thick white in the total white of the egg irrespective of whether the differences in this proportion were the result of the natural variation between newly-laid eggs or were caused by the effects of time and temperature after laying.

5. It is concluded that the relation between rigidity and the proportion of thick white cannot be explained by the depolymerization of a simple ovomucin network.

INTRODUCTION

On the average, nearly one-half of the white of a newly-laid egg consists of a gel (the thick white) that is interposed between two liquid fractions (the outer thin white and the inner thin white). The disposition of the three fractions within the egg is depicted in Fig. 1 (see GROSSFELD[1]; ROMANOFF AND ROMANOFF[2]). The thick white is not a homogeneous gel; to the naked eye it appears to consist of a transparent phase separated by a series of translucent bands with their centres about 1 mm apart. Microscopically, a band is seen to be a stratum of closely-packed, parallel fibres or sheets whereas few fibres can be seen between the bands (HERINGA AND VAN KEMPE

References p. 250.

VALK[3]; ALMQUIST AND LORENZ[4]; MCNALLY[5]; MORAN[6]; SCHAIBLE, MOORE AND DAVIDSON[7]; MORAN AND HALE[8]).

It is usually assumed that the thick white is held together by the microscopic fibres, and that the transparent phase is a liquid resembling the thin white (see ROMANOFF AND ROMANOFF[2]). The fibres themselves are usually believed to be composed of swollen ovomucin (ALMQUIST AND LORENZ[4]; MCNALLY[5]; ALMQUIST, GIVENS AND KLOSE[9])—the ovomucoid-β of MEYER[10]—although it has been suggested by HERINGA AND VAN KEMPE VALK[3] and by MORAN AND HALE[8] that they may also contain keratinous material. After the egg is laid, the volume of thick white diminishes progressively, and the volume of thin white increases correspondingly, at a rate depending on the temperature at which the egg is kept. Although these changes in volume have been studied by many workers, little is known about the mechanical properties of the gel, and the changes which they undergo when the egg is kept. The proportion of thick white in the total white is an inherited characteristic (LORENZ, TAYLOR AND ALMQUIST[11]; KNOX AND GODFREY[12]) but it does not seem to be known

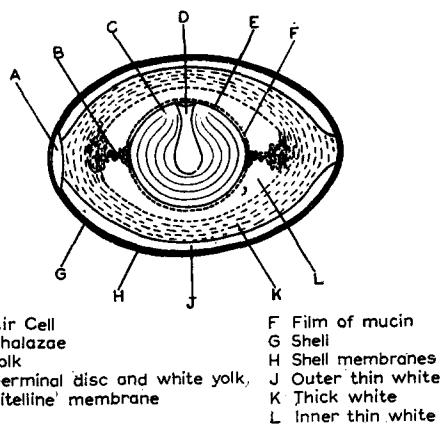

Fig. 1. Structure of the hen's egg as shown by a section through the long axis.

exactly what part it plays in embryonic development. It has been suggested by NEEDHAM[13] that changes in the thick white may be responsible for the fall in hatchability with storage time before incubation.

This paper describes a study of the mechanical properties of the thick white using a method devised by FREUNDLICH AND SEIFRIZ[14] in which the displacement of a minute nickel sphere inserted in a gel is measured in a magnetic field. This micro-method seemed to be particularly suitable for the study of a heterogeneous gel. For stresses of short duration, it was found that the displacement of a particle placed in either a band or the transparent phase was elastic in character. The thick white, therefore, is not an entanglement network like the mitin gel secreted by the hagfish (FERRY[15]) which owes its form solely to fibres of microscopic dimensions, but is a weak gel interpenetrated by a system of microscopic, elastic fibres. It was also found that although the two types of structure in the thick white had different rigidities, both of them were related quantitatively to the proportion of thick white in the total white irrespective of whether the differences in this proportion were the result

References p. 250.

of the natural variation between newly-laid eggs or were caused by keeping eggs for different periods at different temperatures.

The magnetic particle method provides a relative measure of the rigidity of different gels in arbitrary units. In the present work, the conversion of these values into absolute units was effected indirectly by means of a series of parallel measurements on gelatin gels of different concentrations using, on the one hand, the magnetic particle method and, on the other, one of the classical methods that enabled the rigidity to be measured in c.g.s. units.

MATERIALS AND METHODS

Eggs

Hen eggs of known history were obtained from various sources. Some of them were examined at intervals after keeping them for short periods at 20° or for long periods at 0°. In order to reduce evaporation, eggs kept at 0° were first dipped for a few seconds in a white mineral oil and were kept in air of a relative humidity of 80 %; the average weight loss was about 0.22 % per month.

Gelatin

A batch of commercial leaf gelatin was finely powdered and thoroughly mixed. Its water and ash content were 10.5 and 0.16 % respectively, and the pH of a 0.5 % solution in water was 5.64.

The modulus of rigidity

The modulus of rigidity (G) of the gelatin gels was determined by subjecting them to torsion between concentric cylinders using the type of instrument described by OWENS AND MACLAY[16] and OWENS, PORTER AND MACLAY[17]. Many forms of this method have been used since the original work of SCHWEDOFF[18].

A stainless steel cylinder, 10 cm long and 1.25 cm in diameter, was suspended concentrically in a glass cylinder, 4 cm in diameter, by a steel wire, 20 cm long, from a torsion head. A short extension of the steel cylinder, 0.75 cm in diameter, projected above the top of the glass cylinder and carried a small chuck to grasp the wire and a small mirror. The lower end of the steel cylinder dipped in a layer of mercury to reduce end effects. A fixed volume of gelatin solution was poured into the space between the cylinders and allowed to set under standard conditions (*v.i.*) beneath a layer of medicinal paraffin. The torsion head was rotated through an angle of 10° at a speed of 0.042 rev./min. The angle through which the steel cylinder turned was measured by the deflection of a light beam by the mirror; the length of the optical lever was 1 metre. A reading was taken immediately after the movement of the torsion head had ceased, and at intervals of 1 min thereafter for 10 min. The measurements were made in a constant temperature room at 15°.

In a number of instances, the proportionality between stress and strain was confirmed by similar experiments in which the torsion head was turned through only 5°. Thinner wires with smaller torsional moments were used with the weaker gels in order not to strain them unduly; this procedure did not cause an appreciable error. The modulus of rigidity (G) of the gel is given by equation (1); all values are in c.g.s. units (SCHWEDOFF[18]).

References p. 250.

$$G = \frac{N}{4\pi h}\left(\frac{1}{R_1^2} - \frac{1}{R_0^2}\right)\frac{\delta}{\omega} \qquad (1)$$

where N = torque per unit twist of the wire
h = height of the gel
R_0 and R_1 = radii of the outer and inner cylinders respectively
δ = $\phi - \omega$, where ϕ and ω are respectively the angles through which the torsion head and the inner cylinder turn.

The torque per unit twist (N) of the different wires (ranging in diameter from 0.18 to 0.34 mm) was determined in the usual manner by measuring the period of oscillation of a circular brass disc suspended by them.

The magnetic particle method

The procedure was similar to the one used by FREUNDLICH AND SEIFRIZ[14]. The chief differences lay (a) in the use and method of mounting of a much smaller electromagnet, (b) a device to restrict evaporation from the gel, and (c) the use of a Ramsden eyepiece in place of a micro-ocular scale. A Chambers micro-manipulator (E. Leitz) was used to introduce the nickel particle into the gel. The gel itself was contained in a small rectangular Perspex box mounted on a square Perspex platform which fitted into the mechanical stage of the microscope. In order to reduce evaporation, the box was fitted with a Perspex shutter which carried strips of moist cotton wool on its under surface. The shutter was kept closed during the setting of gelatin gels, and opened to a slit during manipulations.

The nickel powder kindly given to us by the Mond Nickel Company Limited consisted of spherical particles. A particle of the desired diameter (about 19 μ) was selected and placed from 0.5 to 1 mm below the surface of the gel with the aid of two glass micro-manipulator needles. The sharp horizontal tip of the magnet was inserted to the same depth in the gel, and moved towards the particle until the distance (r) between point and particle was a little less than 2 mm; measurements were started 20 min later. The coil of the electromagnet (length and diameter both 4 cm; resistance 0.3 ohms) consisted of 220 turns of enamelled copper wire (diameter 1.3 mm) and was activated by a 2 V accumulator. The magnet was turned from a single billet of soft iron. It was 1.5 cm in diameter over a length of 26 cm. It then decreased continuously in diameter and bent through two right angles so that the slender tip (1 cm long) was parallel with but 1 cm distant from the main arm of the magnet. The coil was mounted on the main arm just before its diameter started to decrease (distance between end of tip and nearer face of coil, 7 cm). The main arm of the electromagnet passed horizontally through a brass frame equipped with a vernier measuring device and a graduated drum attached to a spring-loaded, fine-threaded screw so that the electromagnet could be moved backwards or forwards in the frame. The frame itself was mounted on a vertical rack and pinion. The combination of a mechanical stage on the microscope and a small electromagnet that could be moved smoothly in a vertical or in a measured horizontal direction greatly aided manipulation.

The movement of the particle in the magnetic field was consistent with a visco-elastic deformation of the gel. When the field was switched on, a relatively rapid displacement (Δ) was succeeded abruptly by a much slower movement. It was not difficult to follow the moving particle with the hair of the Ramsden eyepiece, and

References p. 250.

to switch off the field at the end of the initial displacement (Δ). When this was done with either gelatin gels or thick white, the particle returned to its original position when the field was switched off. After measuring r and Δ in this manner, the distance between the particle and the point of the magnet was decreased by 80 μ, and another pair of values was obtained. This procedure was repeated until the particle ruptured the gel when the field was applied, and attached itself to the point of the magnet. The measurements were made at 15°. Judging by the displacements in gelatin gels, the small electromagnet exerted much the same force on the particle as the larger electromagnet of FREUNDLICH AND SEIFRIZ[14].

Estimation of the fractions of egg white

The amount of thick white is usually estimated by some form of screening (HOLST AND ALMQUIST[19]; LORENZ AND ALMQUIST[20]; MORAN[21]). The method described below is rapid, and gives consistent results when a standardised procedure is used.

A three-sided, shallow square tray of brass wire gauze was placed on a Petri dish and the two were weighed together. The width of the square apertures in the gauze and the diameter of the wire were 1.36 and 0.46 mm respectively. The egg was broken into the tray, which was then tilted gently to and fro for 1 min to hasten drainage of the outer thin white. The remainder of the egg on the tray was slid on to a clock glass, and the tray and dish were weighed again to obtain the weight of outer thin white. The sac (thick white enclosing inner thin white) surrounding the yolk on the clock glass was cut in several places to liberate the inner thin white, and the procedure described above was repeated with a clean tray and dish to obtain the weight of inner thin white. The yolk and thick white remaining on the tray were slid into an egg separator, and the thick white and chalazae were passed through the slit into a weighed beaker to obtain the weight of thick white. Scalpels or rubber policemen were used to aid the various transfers of material. The thin white drains readily through the gauze if the tray is washed with running water immediately before use and dried with a cloth. The tray was 14 × 14 cm square.

It is known (HOLST AND ALMQUIST[19]; KNOX AND GODFREY[22]; HALNAN AND MORAN[23]) that the proportion of thick white varies greatly in the newly-laid eggs of different hens (although there is a much smaller variation in this respect between the eggs laid by a given hen). The extent of the variation is shown by the values in Table I, which were obtained from two samples, each of 20 eggs, drawn at random

TABLE I

THE RELATIVE PROPORTIONS OF THE THREE FRACTIONS OF THE WHITE IN TWO RANDOM SAMPLES (n = 20) FROM 360 NEWLY-LAID EGGS

Sample	*Fraction of white*	*Range (%)*	*Mean (%)*	*Standard deviation (±)*	*Standard error of mean (±)*	*Coefficient of variation (%)*
A	Outer thin	13.9–31.8	23.31	4.37	0.98	18.8
	Thick	35.4–54.2	46.25	4.64	1.04	10.0
	Inner thin	25.1–36.4	30.45	3.37	0.75	11.1
B	Outer thin	14.5–38.9	22.54	5.64	1.26	25.0
	Thick	40.3–53.5	46.49	3.59	0.80	7.7
	Inner thin	18.1–39.0	30.97	4.41	0.99	14.2

References p. 250.

from a batch of 360 eggs that were less than 24 h old. The average weight of the white of a newly-laid egg is about 33 g.

The consistency of the method can be judged from the fact that statistical analysis showed that for no fraction was there a significant difference between the two samples for mean or variance, nor for the linear regression coefficient for inner thin white on outer thin white. One egg in sample B gave an extreme reading but such an outlier could be expected by chance in 40 % of such samples of 20 eggs.

COMPARATIVE MEASUREMENTS ON GELATIN GELS

The measurements were made on gelatin gels with a similar range of rigidity to that encountered in thick white. In terms of gelatin concentration, this range was about 0.4 to 1.0 %. As the properties of gelatin gels are influenced by their thermal history (FERRY[24]), a strictly standardised procedure was adopted for their preparation. The gelatin was allowed to swell for 2 h in water at 15° before heating the mixture to 80°. The solution was then rapidly cooled to 10°, and immediately poured between the concentric cylinders or into the Perspex box at 15°. All measurements were made exactly 24 h later. Concentrations are given in terms of dry gelatin, and were determined by measuring the dry weight of the gel at the end of the experiment.

Torsional measurements

The modulus of rigidity was obtained from equation (1) by substituting in it the values of δ and ω observed immediately after the movement of the torsion head had ceased. As is usual in static measurements of this type, there was a slow relaxation of stress after the initial rapid deformation which was made evident by an increase in the value of ω with time. This relaxation is usually attributed to the breaking of cross-linkages in the elastic structure of the gel (HATSCHEK[25]; HATSCHEK AND JANE[26]; MILLER, FERRY, SCHREMP AND ELDRIDGE[27]). It was only in the weakest gels that relaxation was sufficiently rapid to impair the accuracy of the initial reading. Examples of the rate of relaxation in four of the gels after movement of the torsion head had ceased at $t = 0$ minutes are set out in Table II (the same wire was not used in every case).

TABLE II

CHANGE OF ω WITH TIME ($\phi = 0.1745$ RADIANS)

g Gelatin in 100 g gel	*Value of ω (radians) at time t (minutes)*					
	0	*1*	*2*	*3*	*4*	*10*
0.955	0.085	0.087	0.087	0.088	0.088	0.089
0.752	0.111	0.116	0.117	0.118	0.118	0.120
0.651	0.094	0.100	0.103	0.104	0.104	0.108
0.561	0.132	0.147	0.148	0.149		0.154

The values of the modulus of rigidity (G) of the gels are set out in Table III, and the values of log G are plotted against gelatin concentration (c) in Fig. 2. Little is known about the mechanical properties of such weak gels. LEICK[28], SHEPPARD AND SWEET[29], FERRY[30] and others have found that over a concentration range of

References p. 250.

about 2 to 30 % of gelatin, the rigidity is proportional or nearly proportional to the square of the concentration. Fig. 2 shows that between 0.5 and 1.0 % of gelatin, the rigidity is an exponential function of the concentration and proportional to a much higher power of the concentration than the square (see also FREUNDLICH AND SEIFRIZ[14]; EDELMAN AND REBINDER[31]; BUNGENBERG DE JONG, VAN DEN BERG AND VERHAGEN[32]). A probable explanation of the difference between the two concentration ranges is that as the concentration falls below about 1 %, a rapidly increasing proportion of the gelatin chains are not cross-linked at all, and do not contribute to the rigidity of the gel (BUNGENBERG DE JONG, VAN DEN BERG AND VERHAGEN[32]).

TABLE III

MODULUS OF RIGIDITY OF GELATIN GELS AT 15°

g Gelatin in 100 g gel	0.955	0.930	0.896	0.892	0.852
G (dynes/cm^2)	185	187	153	155	95
g Gelatin in 100 g gel	0.752	0.710	0.651	0.592	0.561
G (dynes/cm^2)	38.5	30.0	13.5	8.5	5.0

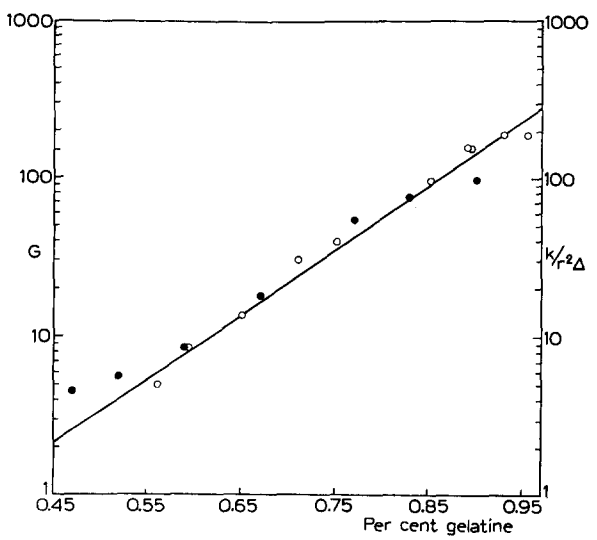

Fig. 2. Comparison of the modulus of rigidity (G dynes/cm^2) of gelatin gels measured by the torsional method (○) with values of ($k/r^2\Delta$) measured by the magnetic particle method (●).

Magnetic particle measurements

We found, as did FREUNDLICH AND SEIFRIZ[14], that $r^2\Delta$ for a given set of paired values was reasonably constant when the limitations of the method were taken into account. The mean value of ($1/r^2\Delta$) was therefore taken as a measure of the modulus of rigidity although apparently, according to FREUNDLICH AND SEIFRIZ[14], Δ would be expected to vary inversely with a higher power of r than the square. A typical set of values is shown in Table IV. With gelatin gels, about 10 to 20 sec were required for the particle to travel the distance Δ.

References p. 250.

TABLE IV

RELATION BETWEEN DISTANCE (r) AND DISPLACEMENT (Δ) FOR A 0.59% GELATIN GEL

r (cm)	$\Delta \times 10^4$ (cm)	$r^2\Delta \times 10^5$
0.160	17.6	4.51
0.152	22.4	5.17
0.144	27.2	5.63
0.136	32.0	5.92
0.128	38.5	6.31
0.120	43.0	6.19
0.112	46.5	5.81
0.104	53.0	5.72
0.096	64.0	5.90
0.088	77.0	5.96
0.080	95.0	6.08
0.072 (yield point)		

Nickel particles ranging in diameter from 18 to 20 μ were used. Mean values of ($r^2\Delta$) were corrected to a common diameter of 19 μ by means of the relation established by FREUNDLICH AND SEIFRIZ[14]; namely, that ($r^2\Delta$) is directly proportional to the square of the diameter (d) of the particle. As shown below, we found that a similar relation held.

d (cm)	$r^2\Delta \times 10^5$	$d^2/r^2\Delta$
0.0029	3.29	0.256
0.0014	0.72	0.272

It will be appreciated that the magnetic particle method has serious limitations when differences between gels are small. Fortunately, the rigidity of both gelatin gels and thick white differed greatly within the respective ranges investigated. The values of ($1/r^2\Delta$) for gelatin gels are set out in Table V.

TABLE V

VALUES OF ($1/r^2\Delta$) FOR GELATIN GELS AT 15°

g Gelatin in 100 g gel	0.90	0.83	0.77	0.67	0.59	0.52	0.47
$(1/r^2\Delta) \times 10^{-5}$	1.72	1.33	0.97	0.315	0.151	0.100	0.080

The logarithms of ($k/r^2\Delta$), where $k = 55.6 \cdot 10^{-5}$, are plotted against gelatin concentration (c) in Fig. 3. It will be seen that there is a reasonable agreement with the values determined by the torsional method although, in fact, the rate of increase of log G with unit per cent increase in c is significantly greater ($P < 0.05$) than the corresponding increase in log ($k/r^2\Delta$). The correlation coefficients (r) and the linear regression equations are given below

$$\log_{10} G = -1.502 + 4.07c \quad (r = +0.994^{***})$$
$$\log_{10} (k/r^2\Delta) = -0.996 + 3.40c \quad (r = +0.991^{***})$$

where*** denotes the 0.1 % level of significance.

References p. 250.

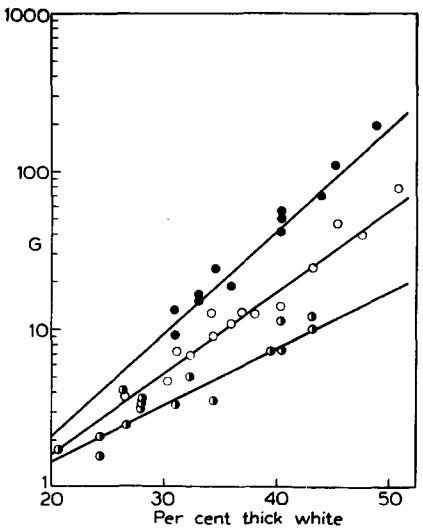

Fig. 3. Relation between the rigidity of the thick white and the proportion of thick white. Particle in fibres pulled at right angles (●) and parallel (○) to their direction. Particle in transparent phase of thick white (◑).

When the agreement over the range is considered, it seems justifiable to translate the results of the magnetic particle method into c.g.s. units by means of the relation:

$$G \text{ (dynes/cm}^2\text{)} = k/r^2\Delta$$

where k for the experimental conditions described in this paper is $55.6 \cdot 10^{-5}$.

MEASUREMENTS ON THICK WHITE

The three fractions of the white of an egg were separated and weighed. The Perspex box was filled with a portion of the thick white in such a manner that some of the bands were parallel with one of the sides of the box. A nickel particle was inserted in either the transparent phase between the bands or well within the microscopic fibres of one of the bands. Thereafter, the procedure was the same as that described for gelatin gels except that when a particle was situated within a band it was pulled in a direction either at right angles or parallel to that of the fibres. The direction of pull proved to have no appreciable effect on the behaviour of a particle situated in the transparent phase. A few experiments showed that the thin white was practically devoid of elastic properties.

Mean values of $(1/r^2\Delta)$, after being corrected to a common particle diameter of 19 μ, were converted into absolute units in the way described above. Values of log G are plotted against the percentage (p) of thick white in the total white in Fig. 3. For each of the sets of measurements upon particles either in the bands or in the transparent phase, the relation between log G and p is approximately linear, and least closely so in the case of the transparent phase between the bands. The correlation coefficients of log G with p, and the regression equations are set out below.

References p. 250.

Particles in the bands, pulled at right angles to direction of fibres

$$\log_{10} G = -1.026 + 0.0658\,p \qquad (r = +0.977^{***})$$

Particles in the bands, pulled parallel to direction of fibres

$$\log_{10} G = -0.833 + 0.0516\,p \qquad (r = +0.973^{***})$$

Particles in transparent phase

$$\log_{10} G = -0.551 + 0.0363\,p \qquad (r = +0.940^{***})$$

where*** denotes the 0.1% level of significance.

The most striking feature of the results is that the mechanical properties of the structures in the thick white seem to be largely independent of the origin and treatment of the eggs except insofar as these have determined the proportion of thick white in the total white. For this work, eggs with different proportions of thick white were obtained in three ways: by taking advantage of the natural variation between newly-laid eggs, by keeping eggs up to 4 weeks at 20°, or up to 6 months at 0°. The results shown in Fig. 3 were obtained with roughly equal numbers of these three types of egg, newly-laid eggs predominating when the percentage of thick white exceeded 40, and eggs kept at 20° when the percentage was less than 30.

The three lines in Fig. 3 tend to converge towards a common value. For a rigidity of 1 dyne/cm^2, the corresponding proportions of thick white given by the extrapolation of the three regression equations are 15.5, 16.3 and 15.3%. The convergence of the lines is consistent with the visible changes which take place in the bands when eggs are kept. After a time, depending on the temperature, the bands can no longer be seen by the naked eye; microscopically, the fibres in the bands appear to be less distinct, less numerous and more loosely packed. These changes gradually become more pronounced, and when p falls below roughly 30%, the bands have been replaced by bundles of scattered fibres. It is evident that the structure of the bands weakens more rapidly than the structure of the gel between the bands but it is doubtful if this can be taken as an indication of a chemical difference between the fine structure of the two phases.

It might be expected that the direction of pull would have an effect on the elastic displacement of a particle embedded in a stratum of parallel fibres. There is little doubt that the fibres are highly swollen for even in an egg containing a high proportion of thick white, the rigidity of the fibres at right angles to their direction corresponds to that of a gel containing only about 1% of gelatin. For ease of comparison, the concentrations of gelatin corresponding to various proportions of thick white are set out in Table VI; the values were obtained by means of the regression equations given above.

TABLE VI

THE CONCENTRATION OF GELATIN IN GELS OF THE SAME RIGIDITY AS THICK WHITE

Percentage of thick white	*Equivalent gelatin concentration (% w/w)*	
	Transparent phase	*At right angles to fibres*
50	0.67	0.96
40	0.56	0.77
30	0.45	0.57
20	0.35	0.38

References p. 250.

RUPTURE OF THE GELS

FREUNDLICH AND SEIFRIZ[14] denoted the displacement of the particle observed in the measurement immediately before the one in which rupture of the gel occurred as the elastic limit of the gel. This limit does not correspond to the stress above which plastic deformation can first occur (for all the gels can exhibit visco-elastic characteristics if the magnetic field is applied for long enough) but to the stress above which a predominantly elastic deformation passes rapidly into a fully plastic one. The values of Δ max (in μ) observed with both gelatin gels and thick white are plotted against the corresponding values of log G in Fig. 4. The values for gelatin gels lie near to a smooth curve. The values for thick white are much more widely scattered but in spite of this it is evident that the thick white structures can be strained without rupture to a much greater extent than a gelatin gel of a similar rigidity, and that the difference becomes more pronounced as the rigidity diminishes. At comparable values of G, the structures of the bands and that of the transparent phase rupture, as far as can be judged, when much the same value of Δ max is exceeded. Although FREUNDLICH AND SEIFRIZ[14] were chiefly concerned with gelatin gels, they made one experiment with the thick white of an egg. They found that it had an elasticity equivalent to that of a 1.1% gelatin gel but possessed a considerably greater value of Δ max.

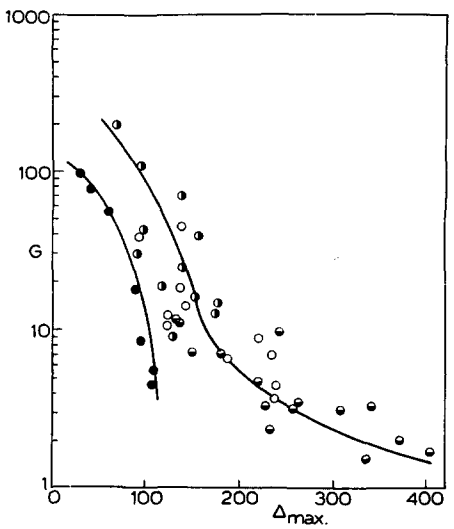

Fig. 4. Relation between the rigidity and the maximum displacement, Δ_{max} (in μ), of gelatin gels (●) and thick white. Particle in fibres pulled at right angles (◑) and parallel to their direction (○). Particle in transparent phase of thick white (◒).

DISCUSSION

The magnetic particle method can yield little information about the more complicated properties of gels such as relaxation or creep; nor is it accurate enough to distinguish small differences in the chief characteristic of a gel—its rigidity. Nevertheless, the method can provide valuable information about weak, natural gels which, like the thick white, cannot be studied easily by the usual methods. A serious drawback of the method is the skill which has to be acquired before a particle can be introduced

References p. 250.

smoothly beneath the surface of the gel. In one instance, this difficulty has been avoided in an ingenious manner; CRICK AND HUGHES[33] studied the mechanical properties of cytoplasm after cells in tissue culture had been allowed to phagocytose small magnetic particles.

The present work has shown that the thick white, contrary to the usual belief, is a weak gel in which bands of microscopic fibres are embedded, and moreover, that its gradual shrinkage is accompanied by a rapid decrease in the rigidity of the two phases within it. From the general theory of polymer structure (FLORY[34,35]; FLORY AND REHNER[36]; GUHT, JAMES AND MARK[37]), it would seem that the observed changes in rigidity are best explained by a gradual depolymerization of cross-linked, three-dimensional networks existing in both the fibres and the transparent phase of the thick white. It is difficult to see, however, why a simple depolymerization should be accompanied by a gradual decrease in the volume of the thick white. On the contrary, it would be expected that as rigidities decreased, the volume would not alter or would even increase by absorption of thin white until the stage was reached when the number of cross-linkages had fallen below the minimum necessary for gelation, and that then there would be a relatively abrupt transition of the whole of the gel into a liquid. On the other hand, the progressive shrinkage of the thick white cannot be accepted as another example of the syneresis which occurs in such relatively simple gels as those of dilute silica (FERGUSON AND APPLEBY[38]) or di-benzoyl cystine (WOLF AND RIDEAL[39]) since it would be expected that the spontaneous contraction of a gel with the liberation of the dispersion medium would be associated with the progressive formation of more cross-linkages and a corresponding increase in rigidity. We have, in fact, studied the mechanical properties of dilute silica gels (by the application of torsion between concentric cylinders) and found, as would be expected, that the course of syneresis is accompained by a marked and continuous increase of rigidity.

It is possible to reconcile a decreasing rigidity with a decreasing volume if it is assumed that depolymerization is accompanied by a continuous change in the chemical composition of the surviving networks. It is known (FLORY AND REHNER[36]; FLORY[40]) that cross-linked networks swell in suitable liquids to an equilibrium volume which is characteristic of the network and the liquid. On this basis, it is suggested that the progressive shrinkage of the thick white is the result of a corresponding decrease in the equilibrium swelling volumes of a series of chemically changed networks. Thick white contains a much higher proportion of ovomucin than thin white, and there seems to be little doubt that this is why the thick white is a gel and the thin white is not since this is the only major difference in chemical composition between them. It is known, however, that there is an interaction between lysozyme and ovomucin (HAWTHORNE[41]), and there is reason to believe that changes in this interaction may be responsible for the changes which occur in the thick white when the egg is kept (HAWTHORNE[41]; FEENEY, DUCAY, SILVA AND MACDONNELL[42]; COTTERILL AND WINTER[43]; WILCOX[44]). If the networks in the thick white of the newly-laid egg are composed of ovomucin chains associated with or cross-linked by lysozyme molecules, a gradual dissociation or hydrolysis of the complex would correspond with the chemical change associated with depolymerization that was postulated above.

An interesting point arose from an examination of the changes which occurred in the relative proportions of the three fractions of the white when eggs were kept

References p. 250.

at 0°. It was evident that the liquid lost by the shrinking thick white was distributed in a very one-sided manner, and furthermore, it seemed probable that the envelope of thick white as a whole was in a contractile state. Results obtained with oiled eggs kept in air of a relative humidity of 80% at 0° are set out in Table VII. Each of the three samples consisted of 20 eggs drawn at random (as in Table I) from an initial batch of 360 eggs. Statistical analysis showed that the effect of time on the relative proportions of the three fractions was highly significant. Similar results have been obtained in other experiments with both oiled and unoiled eggs, and similar values have been reported for unoiled eggs by MORAN[21].

TABLE VII

THE EFFECT OF KEEPING OILED EGGS AT 0° AND 80% RELATIVE HUMIDITY

Time (weeks)	*Average weight of total white (g)*	*Average percentage of total white as:*		
		Outer thin white	*Thick white*	*Inner thin white*
0	33.0	22.7	46.4	30.9
12	31.9	45.8	37.4	16.8
24	30.6	52.9	31.5	15.6

It will be seen from the Table that all the liquid lost by the thick white is gained by the outer thin white, and furthermore, that an actual transfer of the inner thin white to the outer thin white apparently takes place at the same time. This transfer is too large to be accounted for by the movements of water which are known to take place within the egg. Water normally moves from the white in two directions when the egg is kept—outwards to the atmosphere and inwards to the yolk. The water lost by the white to a single yolk at 0° is about 5 mg per day (SMITH[45]) while the water lost by evaporation from an oiled egg kept at 0° in air of a relative humidity of 80% is, on the average, about 4 mg per day. These losses are in reasonable agreement with the changes in the observed weights of the total white given in the second column of Table VII, and are too small—in whatever manner they are distributed amongst the three fractions—to account for the changes with time in the relative proportions of the three fractions. Since the small osmotic differences that exist between the three fractions (SMITH[45]) would favour the transfer of water to the inner thin white, it is difficult to resist the conclusion that at 0° the envelope of thick white as a whole is in a contractile state, and that it slowly squeezes inner thin white outwards through its own structures together with the liquid lost by its own shrinkage.

ACKNOWLEDGEMENTS

The authors are pleased to express their thanks to the Mond Nickel Company Limited for the gift of a sample of nickel powder. Mr. N. R. KING assisted in the experimental work described in this paper. The work was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

References p. 250.

REFERENCES

1 J. GROSSFELD, *Handbuch der Eierkunde*, Julius Springer, Berlin, 1938.
2 A. L. ROMANOFF AND A. J. ROMANOFF, *The Avian Egg*, John Wiley and Sons, Inc., New York, N.Y., 1949.
3 G. C. HERINGA AND S. H. VAN KEMPE VALK, *Proc. Acad. Sci. Amsterdam*, 33 (1930) 530.
4 H. J. ALMQUIST AND F. W. LORENZ, *U.S. Egg Poultry Mag.*, 38 (4) (1932) 20.
5 E. MCNALLY, *Proc. Soc. Exptl. Biol.*, 30 (1932–33) 1254.
6 T. MORAN, *Dept. Sci. Ind. Research (Brit.) Index Lit. Food Invest.*, 1934, p. 52.
7 P. J. SCHAIBLE, J. M. MOORE AND J. A. DAVIDSON, *U.S. Egg Poultry Mag.*, 41 (12) (1935) 38.
8 T. MORAN AND H. P. HALE, *J. Exptl. Biol.*, 13 (1936) 35.
9 H. J. ALMQUIST, J. W. GIVENS AND A. KLOSE, *Ind. Eng. Chem.*, 26 (1934) 847.
10 K. MEYER, *Cold Spring Harbor Symposia Quant. Biol.*, 6 (1938) 91.
11 F. W. LORENZ, L. W. TAYLOR AND H. J. ALMQUIST, *Poultry Sci.*, 13 (1934) 14.
12 C. W. KNOX AND A. B. GODFREY, *Poultry Sci.*, 19 (1940) 291.
13 J. NEEDHAM, *Biochemistry and Morphogenesis*, Cambridge University Press, Cambridge, 1942.
14 H. FREUNDLICH AND W. SEIFRIZ, *Z. phys. Chem.*, 104 (1923) 233.
15 J. D. FERRY, *J. Biol. Chem.*, 138 (1941) 263.
16 H. S. OWENS AND W. D. MACLAY, *J. Colloid Sci.*, 1 (1946) 313.
17 H. S. OWENS, O. PORTER AND W. D. MACLAY, *Food Ind.*, 19 (1947) 606.
18 T. SCHWEDOFF, *J. phys.*, [2], 8 (1889) 341.
19 W. F. HOLST AND H. J. ALMQUIST, *Hilgardia*, 6 (1931) 49.
20 F. W. LORENZ AND H. J. ALMQUIST, *U.S. Egg Poultry Mag.*, 40 (11), (1934) 30.
21 T. MORAN, *J. Soc. Chem. Ind.*, 56 (1937) 96T.
22 C. W. KNOX AND A. B. GODFREY, *Poultry Sci.*, 13 (1934) 18.
23 E. T. HALNAN AND T. MORAN, *Dept. Sci. Ind. Research (Brit.) Index Lit. Food Invest.*, 1936, p. 44.
24 J. D. FERRY, *Advances in Protein Chem.*, 4 (1948) 1.
25 E. HATSCHEK, *Kolloid-Z.*, 28 (1921) 210.
26 E. HATSCHEK AND R. S. JANE, *Kolloid-Z.*, 39 (1926) 300.
27 M. MILLER, J. D. FERRY, F. W. SCHREMP AND J. E. ELDRIDGE, *J. Phys. Chem.*, 55 (1951) 1387.
28 A. LEICK, *Ann. Physik.*, [4], 14 (1904) 139.
29 S. E. SHEPPARD AND S. S. SWEET, *J. Am. Chem. Soc.*, 43 (1921) 545.
30 J. D. FERRY, *J. Am. Chem. Soc.*, 70 (1948) 2244.
31 L. I. EDELMAN AND P. A. REBINDER, *C. A.*, 45 (1951) 6008.
32 H. G. BUNGENBERG DE JONG, H. J. VAN DEN BERG AND H. J. VERHAGEN, *Proc. Acad. Sci. Amsterdam*, B 55 (1952) 1, 14.
33 F. H. C. CRICK AND A. F. W. HUGHES, *Exptl. Cell Research*, 1 (1950) 37.
34 P. J. FLORY, *J. Am. Chem. Soc.*, 63 (1941) 3083, 3091, 3096.
35 P. J. FLORY, *Chem. Revs.*, 35 (1944) 51.
36 P. J. FLORY AND J. REHNER, *J. Chem. Phys.*, 11 (1943) 512, 521.
37 E. GUTH, H. M. JAMES AND H. MARK, *Advances in Colloid Sci.*, 2 (1946) 253.
38 J. FERGUSON AND M. P. APPLEBY, *Trans. Faraday Soc.*, 26 (1930) 642.
39 C. G. L. WOLF AND E. K. RIDEAL, *Biochem. J.*, 16 (1922) 548.
40 P. J. FLORY, *J. Chem. Phys.*, 18 (1950) 108.
41 J. R. HAWTHORNE, *Biochim. Biophys. Acta*, 6 (1950) 28.
42 R. E. FEENEY, E. D. DUCAY, R. B. SILVA AND L. R. MACDONNELL, *Poultry Sci.*, 34 (1952) 679.
43 O. COTTERILL AND A. R. WINTER, *Poultry Sci.*, 34 (1955) 679.
44 F. H. WILCOX, *Poultry Sci.*, 34 (1955) 1170.
45 A. J. M. SMITH, *Dept. Sci. Ind. Research (Brit.) Index Lit. Food Invest.*, 1931, pp. 162, 165.