Suitability of hen eggs for incubation in the fresh state and after storage

Review based on the study of 198 references
by V.V. Rol'nik

Translated by L. Weiss-Vladimirov
With an additional bibliography of 56 titles

1977
Centre for Agricultural Publishing
and Documentation
Wageningen, the Netherlands

Contents

Preface to Russian version

In the Soviet Union, poultry breeding is becoming an industry. New poultry plants and specialized poultry state farms are being built, the work of incubator and poultry breeding stations is being improved, and industrial incubators are planned in which 80-100 million chicks will be hatched annually. Further intensification of industrial poultry farming, for the production of broilers and laying hens, requires hatching of large age-synchronized batches of chicks.

Keba (1971), discussing the advantages of incubating large batches of age-synchronized chicks, stressed the following points: uniform conditions of incubation for embryos at each developmental stage (e.g. start of cooling on a fixed day); better use of biological control data for changes in the incubation schedule; simultaneous emptying and disinfection of incubators; and simplification of work for the staff.

The hatching of a large batch of age-synchronized chicks requires many incubating eggs, sometimes creating the need for long storage of eggs.

High egg quality is known to be a primary condition for good incubation results. The development of the avian embryo outside the mother bird is entirely dependent on the quantity and quality of nutrients present in the egg. Inferior quality of eggs is a major cause of unsatisfactory incubation results and low viability of chicks, while heterogeneity of eggs yields chicks of varying quality.

During storage of eggs for incubation, it is essential to preserve the viability of the embryo, whose development started in the hen's oviduct and stopped temporarily after oviposition. This pause in the embryo's development does not represent, however, total arrest of its vital processes. At this stage, the embryo's condition resembles anabiosis, with extremely reduced rate of metabolism. Prolonged storage of eggs for incubation is often accompanied by a decrease in quality of the egg contents and a reduced capacity for normal development of the embryo, yielding chicks with decreased viability. It may furthermore adversely affect productivity of the hen. The basic requirement for storage of eggs for incubation is, therefore, maintenance as far as possible of properties of the freshly laid egg, and of the embryo's viability.

In recent years there has been a notable increase in research on the qualities of hen eggs for incubation and on changes during storage (Kleimenova, 1972; Otrygan'ev, 1963). The present review attempts to summarize Russian and non-Russian literature on the quality of eggs for incubation in the fresh state and after storage.

1. The egg in the fresh state

1.1. Structure, chemical composition, physical and physico-chemical properties of the egg at oviposition

The egg contents consist on average of 64% white and 36% yolk. The white contains 12% solids, mainly proteins and a small amount of minerals and carbohydrates. The white has four layers: the outer thin layer, the thick layer, the inner thin layer, and the chalaziferous layer which surrounds the yolk membrane and terminates in the chalazae. The chalazae serve to stabilize the egg yolk. The thin white contains very little mucinous fibre, but in the thick white they form a densely interlaced network filled with fluid albumen.

Tsarenko & Tsarenko (1970) observed a higher hatching rate with values close to the average for the ratio of thin to thick white, than with extreme values.

Egg white contains the following proteins (in % by mass of solids):

Ovalbumin	54	Flavoprotein-apoprotein	0,8
Conalbumin	13	Ovo-inhibitor	0,1
Ovomucoid	11	Avidin	0,015
Lysozyme	3,5	Unidentified proteins	
Ovomucin	1,5	(chiefly globulins)	8

The main protein in hen-egg white is ovalbumin. By electrophoresis, it separates into two components, A_1 containing two phosphorus atoms per protein molecule, and A_2 with one phosphorus atom; in the presence of phosphatase A_1 is converted into A_2, and ultimately into component A_3 which is devoid of phosphorus (Lisouskaya & Livanova, 1960). Ovalbumin is an oligoprotein containing all amino acids essential for embryo nutrition. It is the classical example of a protein that, in its natural state, includes 'masked' sulphydryl groups. The ovalbumin molecule has 4 or 5 sulphydryl groups and one sulphydryl ring. The sulphur-containing amino acids cystine and cysteine play an important part in the denaturation of ovalbumin. Smith (1964) showed that during egg storage ovalbumin is converted into a form which is considerably more resistant to denaturation, but in other physical and chemical properties is indistinguishable from ordinary ovalbumin. The molecular weight of ovalbumin is 45 000.

Conalbumin binds iron and thereby makes it unavailable to microorganisms. It is in this respect a homologue of transferrin (serum β-globulin) which forms the livetin fraction of yolk protein, differing from livetin only in the carbohydrate prosthetic group. It has been established that the conalbumin molecule contains 9 peptide chains and no phosphorus or free sulphydryl

groups. The molecular weight of conalbumin is about 8 000. There are 2 conalbumin fractions which in hen-egg white are present in a proportion of roughly 4:1.

Ovomucoid is a glycoprotein that does not coagulate at high temperatures. It has no free sulphydryl groups. Its molecular weight is 28 000. Three fractions have been demonstrated. Ovomucoid contains 20-22% carbohydrates of which 12-14% glucosamines, 4-6% mannose and 1,5% galactose.

Ovomucin is the structural protein of thick white and is responsible for gel quality. Under the electron microscope, the ovomucin molecule appears as a long filament. It consists of a large number of interconnected granules. Disruption of the thick white gel, which occurs with shaking, is explained by the dissociation of these filamentous aggregates (Riley, 1953). It has been postulated that the stability of thick white is due to the ovomucin-lysozyme complex (Cotterill & Winter, 1955). Ovomucin contains much sulphur, more than ovalbumin. Boiling the white of one egg in water yields 10,7 mg hydrogen sulphide. Ovomucin contains 12-14% hexose.

Avidin binds with biotin and thereby inactivates it as a vitamin. At high temperatures, avidin looses its activity. Its molecular weight is 48 000 - 66 000.

Ovo-inhibitor inhibits proteolytic enzymes.

Flavoprotein is the bound form of riboflavin. It is present in minute traces in the egg (3 µg per egg), but is of great importance for tissue respiration of the embryo.

The globulin fraction of egg white consists of three electrophoretically distinct components: G_1, G_2 and G_3. G_3 is lysozyme (Parkinson, 1966).

Lysozyme plays a particular role among egg-white proteins. The bactericidal properties of lysozyme protect the embryo against micro-organisms, at early stages of development, before emergence of the embryo's own immune defence. Lysozyme was discovered by Fleming & Allison (1924). It is an alkaline protein with a molecular weight of 15 000 - 17 000. The molecule contains 8 semi-cystine residues, but no sulphydryl groups which are characteristic for many egg white proteins. Wilcox & Cole (1954) established that eggs from a single laying hen have about the same lysozyme content, whereas it varies among eggs from different hens. The immunity conferred by lysozyme at early developmental stages reduces mortality of chicken embryos in the first 7 days of incubation and thereby maintains hatchability. The hatchability of eggs with high lysozyme content (10.2-13.6% of thick white) was 92%, against 89-90% for eggs low in lysozyme (4.4-8.7% of thick white). Moreover chicks from eggs with high lysozyme levels grew better (Sauter et al., 1970). Analysing differences in chick hatchability, Sauter et al. (1970) found that even the lower lysozyme content is many times greater than that needed for maximum bacteriolytic activity. Apparantly, a high lysozyme content confers to hatchability of eggs in other ways too. A direct genetic link was found between high lysozyme content and egg weight; a high percentage of lysozyme correlates furthermore with a high proportion of thick white, which is an indicator of good egg quality. This

contradicts earlier data (Cotterill & Winter, 1954) denying a correlation between lysozyme activity and egg quality in Haugh units.

The bactericidal activity of egg white lysozyme rises sharply on the third day of incubation. It decreases somewhat by Day 5 but remains above the preincubation level. The increased bactericidal activity of lysozyme, which coincides with rapid development of the cardiovascular system, appears to be related to some metabolite or other, secreted at that time in the embryo and acting towards lysozyme as a coenzyme or inorganic catalyst (Pletsityi & Krasnyanskaya, 1963). Toshkov & Shrova (1970) found increased lysozyme activity on Day 2 of incubation; it dropped on Day 6, afterwards remaining at the same level throughout incubation. The concentration of lysozyme in egg white varies in a somewhat different manner; it increases by Day 2 of incubation, decreases by Day 6, rises again slowly by Day 11, more quickly until Day 15, and then remains constant until the end of incubation.

Egg lysozyme differs among species of bird. Korotkova (1956) and Movchan (1967) examined the antibiotic activity of hen, duck and guinea-hen eggs, and found that the white of hen eggs had the strongest antibiotic properties. The outer layer of white was the most bactericidal, thus providing the avian ambryo with a firm barrier to penetrating bacteria early in development. The more alkaline nature of lysozyme in duck eggs is due to more arginine residue (Jolles et al., 1967).

At a conference in London organized by Perutz (1967), the structure and function of lysozyme was discussed. Lysozyme was the third protein, and the first enzyme, to be thoroughly studied. Lysozymes, of somewhat differing structure, are present not only in egg white of various birds but also in tissues of animals and man, in particular in human milk (where the lysozyme bactericidal activity is three times as high in the hen's egg). Any influence raising the acidity of lysozyme decreases its activity. The inner part of the lysozyme molecule consists mainly of carbohydrates, like the globulin chains. At the conference, Kravchenko from the Soviet Union presented evidence that lysozyme was a transferase.

According to Ball & Cotterill (1971), the reaction of egg-white catalase proceeded in the same way as that of other catalases. Ovalbumin and conalbumin had pratically no catalase activity, which was found to be associated with one of the less studied globulins.

Chung & Stadelman (1961) found that egg-white protein and yolk protein increased by 0.09 g and 0.04 g, respectively, per gram increase in egg weight.

Veitsman et al. (1970) reported differences in amino-acid composition between eggs of different poultry species. There was more lysine (essential for haemoglobin synthesis) in egg white than in yolk; hen and guinea-hen eggs contained more of it than eggs of other species. Quail eggs rank first for phenylalanine and tyrosine content (both required for the synthesis of thyroxine and adrenalin).

The yolk forms an irregular sphere, with an average length of 34 mm, width 32 mm, surface 32.2 cm^2 and volume 17.1 cm^3. In the

centre of the yolk is the latebra, with its neck stretched towards the yolk surface. The oocyte is situated where the latebra reaches the surface. After fertilization, the oocyte forms the blastodisc. The yolk surrounds the latebra in consecutive yellow and white layers. The peripheral yolk lies under the yolk membrane and consists of regressive follicular cells (Iordanov, 1969).

According to Romanoff & Romanoff (1949), the yellow yolk is deposited during daytime till midnight, and the white in the remaining part of the night. Barkovskaya (1954) rejects the distinction between white and yellow yolk, claiming that its colour depends on the feed given to the laying hen. A yolk layer stained with a dye added to the feed remains long without spreading underneath succesive layers. Maurice & Fidanza (1954), who studied the structure of egg yolk by examining its permeability to ^{82}Br, found that after 100-200 h, most of the labelled bromine had remained in the outer layers of yolk. They assumed that the yolk was divided into layers separated by thin membranes of low permeability, and that the layers were 0.3 mm thick. Iordanov (1969) believed that yellow and white yolk layers might be distinguished either by colour, or by colour and histological features. In the former case, it would be the differing amount of pigment contained in the granules that governed the layered appearance of the yolk, and which depends on the pigment contained in the feed of the laying hen. In the latter case, form and size of granules would be considered additionally, and they depend not only on nutrition but on other conditions as well. Vladimirova & Sergeeva (1971) presented interesting data on differences in chemical composition between white and yellow egg-yolk. White yolk contains 86% water, 4.6% proteins, 3.5% lipids, 5.9% carbohydrates and ash; for yellow yolk these percentages are 45, 15, 34 and 6 respectively. They reported that the yolk index [volume fraction of yolk] of eggs from Russian White hens showed seasonal variations. In spring and summer, it was 0.448. in winter 0.480-0.490.

Egg yolk contains the store of nutrients for embryo development. According to Vladimirova & Sergeeva (1968), the yolk of eggs from Russian White hens contained 50.4-53.5% solids, and the white 12. 12.3-14%. the highest hatching rate was observed when solids ranged between 12.5% and 13.3% in the white, and between 52.5 and 53.5% in the yolk. Moist weight and yolk index of turkey eggs was inversely correlated with hatchability (Nestor & Brown, 1968).

Many new data on the microscopic structure and chemical composition of yolk are presented in the monograph by Iordanov (1969). The yolk granule, which is the major morphological element of yolk, is formed in the plasma of the highly specialized ovum. Every yolk granule is composed of the basic substance (liquid) and one or more grains. The basic substance of the yolk granule is a complex crystalloid-colloid system containing in sqlution low-molecular inorganic and organic substances: salts, glucose, free amino acids, some vitamins of the B group; and high-molecular compounds. These form the livetin fraction, including antibodies, enzymes, flavoproteins; and the lipovitellin fraction, including carotenoids, and fat-soluble vitamins. (For the chemical composition of yolk, see Table 1.) Between the granules in yellow and white yolk, there

Table 1. Chemical composition of egg yolk, expressed as mass fraction ($w/10^{-3}$) of solids. After Parkinson (1966). Vitellin and vitellenin are the protein moieties of corresponding lipoproteins and have not been detected in egg yolk in the free state

Component	$w/10^{-3}$
Proteins	
livetins	40-100
phosphoproteins	
vitellin	40-150
vitellenin	80-190
phosvitin	50- 60
lipoproteins	
lipovitellin	160-180
lipovitellenin	120-130
Lipids	
triglycerides	460
phospholipids	200
sterols (mainly cholesterol)	30
Carbohydrates	20
Minerals	20
Vitamins	traces

is no cytoplasm or liquid, while some liquid is present in the peripheral yolk and in the latebra.

The yolk proteins are synthesized outside the oviduct, mainly in the maternal liver, and are transported by the blood to the oviduct plasma.

Livetins, water-soluble yolk proteins, contain 15.1-15.4% nitrogen, 1.8% sulphur, 5.2% tyrosine, 2.1% tryptophan, 3.9% cystine and only traces of phosphorus. There are α-, β- and γ-livetins. Iordanov (1969) mentions that the main constituents of the yolk livetin fraction are related to avian serum proteins, while the presence of antibodies and enzymes in this fraction gives it an important biological function. According to him, the livetin fraction has the highest carbohydrate content, and β-livetin is a true glycoprotein.

Phosphoproteins: vitellin and vitellenin have equal amounts of nitrogen (15.5%) and sulphur (0.9-1.0%), but their phosphorus content differs, 1-2% in vitellin and only 0.3% in vitellenin. The molecular weight of vitellin is about 93 000. Phosvitin contains 12% nitrogen and 10% phosphorus, which makes up roughly two-thirds of total phosphorus in yolk. Its molecular weight is about 36 000. Vorob'ev (1960) studied phosphorus compounds in the hen egg and found that on average yolk contained 0.98% total phosphorus [mass fraction in yolk solids], including 0.142% acid-soluble phosphorus (in white 0.52 and 0.121, respectively). More than half the total phosphorus in yolk, 0.580%, was in the form of phospholipids, compared to only 0.145% in white. The phosphoprotein content in yolk was 0.252%, which was somewhat less than in white (0.268%). Myannik & Nymmisto (1968) established signifi-

cant positive correlations between phosphorus content in yolk and white on the one hand, and the percentage of egg solids ($r = 0.664$) and lipid + carbohydrate content ($r = 0.657$) on the other.

Lipoproteins are complex compounds in which phosphoproteins are bound to phospholipids, and possibly to some neutral triglycerides. Lipovitellin contains 17% bound lipids, 13% nitrogen and 1.5% phosphorus; lipovitellenin contains 36-41% bound lipids (mainly lecithin), 9.9-10.2% nitrogen, 1.5-1.7% phosphorus and 0.6% sulphur. Lipovitellenin resembles a micelle in structure, being composed of protein molecules linked by lipid molecules.

The white yolk has smaller granules than the latebra, whose granules are richer in lipovitellenin (Iordanov, 1969).

The mass concentration of free amino acids in yolk, 41.1 mg/litre, is much higher than in white, 0.15 mg/litre. The concentration of individual free amino acids corresponds to the requirements of the embryo (Fitzsimmons & Waibel, 1968). According to Iordanov (1969), 16 free amino acids are present in yolk, predominantly monoaminodicarboxyl acids, which appear to be a general feature of ova. The amount of free amino acids is fairly constant. During prolonged storage, they diffuse from yolk into white, which itself contains hardly any free amino acids.

Makarova & Bogolyubskii (1969) demonstrated that the content [mass fraction or mass concentration] of nucleic acid in yolk was lower than in white: RNA 0.59 mg/kg [or mg/litre] and DNA 0.39 mg/kg [or mg/litre] in yolk against 0.92 and 0.46 in white. The nucleic acid content is the same in eggs from hens of different breed and different age, but it varies with season of the year, particularly before the onset of oviposition. The amount of DNA in yolk several times exceeds the DNA content of haploid or diploid nuclei. Iordanov (1969) assumed that yolk DNA was a reserve of extranuclear DNA for use in chromosomal DNA in the developing embryo. Yolk contained the following enzymes: tributyrinase, peptidase, catalase, amylase and phosphatidase. It contained no oxidase enzymes.

A large proportion, possibly all, of the yolk lipids (Table 2) are bound to proteins in lipoprotein complexes, but some of them are easily extractable with diethyl ether, and are conventionally called free lipids. The composition and amount of most yolk lipids, including fat-soluble pigments (carotenoids) and vitamins (A, D, E and K) vary with diet and other conditions (Iordanov, 1969).

The fatty-acid composition of yolk lipids depends largely on the types of fat in the diet of the laying hen, but the cholesterol content of yolk is independent of feed composition. Leclerq (1966) reports that most of the fatty-acids from yolk is taken up by the embryo's tissues during development. The author observed that development of the avian embryo depends to a greater extent on maternal nutrition, than is the case with mammalian embryos.

As shown by Moiseeva (1965), the content of cholesterol in yolk is negatively correlated with yolk weight ($r = -0.21$), and with laying performance of pullet chickens hatched from these eggs ($r = -0.15$).

The composition of the phospholipid fraction from yolk lipoproteins is shown in Table 3.

Table 2. Lipid composition of egg yolk. Data from Lea (1962).

Egg yolk			
Moisture (49%)		Solids (51%)	
Triglycerides (65%)	Phospholipids (30%)	Cholesterol (4%)	Carotenoids, vitamins, etc. (traces)
Lecithin lysolecithin (79%)	Phosphatidyl-ethanolamine and lysophosph-atidylethan-olamine (17%)	Sphingo-myelin (2.5%)	Plasmalogens and inositides (traces)

Table 3. Mass fraction ($w/10^{-3}$) of different phospholipids in the phospholipid fraction from lipoproteins of egg yolk. Data from Martin et al. (1963).

Lipoprotein	Sphingomyelin, lysolecithin & lysocepahlin	Lecithin	Cephalin
α-Lipovitellin	0.07	0.75	0.18
β-Lipovitellin	0.07	0.76	0.17
Lipovitellenin	0.08-0.09	0.71-0.76	0.16-0.20
Lipovitellenin extracted with ether	0.07-0.11	0.71-0.74	0.18-0.19

Interesting comparative data on the composition of chicken, turkey, guinea hen and quail eggs have been contributed by Veitsman et al. (1970). Suffice it to mention their data for mass of vitamins in egg yolk: carotene 61.6 μg, vitamin A 192.5 μg, vitamin B-12 0.23 μg. Kolupaeva (1970) pointed out the lipotropic effect of vitamin B-12. Intramuscular administration of vitamin B-12 to laying hens caused a decrease in cholesterol and an increase in phospholipids in yolk, in particular lecithin. Furthermore, when laying hens received a vitamin B-12 concentrate, their eggs contain not only more vitamin B-12 but also more carotene and vitamin A.

Carbohydrates in egg yolk are in the free state as glucose, or protein-bound as a glucoseeminedimannose polymer (Iordanov, 1969). The mass fraction of glucose in yolk is about half that in white.

Table 4 shows that the yolk and white contain somewhat more potassium than sodium, which is a general characteristic of the egg cell. The yolk is rich in phosphorus, most of it in organic compounds. The ratio of anionic to cationic elements is 0.64 in egg white, but in yolk is 1.32. As there are considerably more

Table 4. Mass fractions ($w/10^{-3}$) of mineral elements in ash from egg yolk and egg white. Data from Il'in (1917).

	White	Yolk
Na	174-244	38- 48
K	204-236	66- 74
Ca	12- 21	87- 94
Mg	10- 19	12- 13
Fe	3- 4	8- 10
P	14- 21	279-291
S	5- 10	.
Si	1- 9	.
Cl	238-285	.

solids [and ash] in yolk than in white, the anion-cation ratio for the whole egg is 1.1. Trace elements in yolk and white play an important role in embryonic metabolism. The minerals composition of hen eggs has been studied most comprehensively by Kucherova & Golubev (1970). In addition to elements already reported in the literature, they identified 39 cationic and 7 anionic elements in yolk; 25 cationic and 13 anionic in white; 47 cationic in the shell membrane and 14 cationic in the shell.

Between yolk and white, there are differences in the osmotically effective concentration of substances and in the proportion of cations and anions. The yolk has a higher osmotic pressure and a lower pH than white, which [pH] is of considerable importance for changes during storage and incubation.

Kucherova (1968) assumed that during the first days of incubation, not only water but a variety of minerals migrated from white to yolk, because of the difference in concentration. The permeability of the yolk membrane is enhanced by sodium chloride.

The structure of the yolk membrane has been described in different ways. Shalumovich (1955) detected no less than 5 layers, whereas according to Bellairs et al. (1963) the yolk membrane has two layers consisting mainly of proteins but with different amino-acid composition. Iordanov (1969) reported that electron microscopic examination revealed three layers in the yolk membrane, an outer layer (formed during passage through the oviduct), a middle layer made up of electron-dense fibrous networks, and an inner layer consisting of a single membrane and which in fact is the cell membrane of the ovum. The yolk membrane was highly permeable to macromolecules of proteinaceous type.

The chemical composition of the yolk membrane has not been definitely established. Earlier views that its inner layer had a keratinous or collagenous structure have not been borne out by recent data. The outer layer of the yolk membrane contains ovomucin.

The two shell membranes and the shell protect the embryo from injuries and excessive evaporation, and forms a source of nutrients. The outer shell membrane is tightly joined to the inner surface of the shell, as are also the two shell membranes to each

other, except at the site of the air space. The air space is formed between the shell membranes after oviposition, when the egg contents cool and shrink, and air is sucked in from outside. In hen eggs with relatively thick shells, the shell membranes are thinner and make up 0.6% of total egg mass, compared to roughly 2.2% for turkey eggs which have a thinner shell. Shell membranes are thickest at the blunt end of the egg (Ferdinandov, 1931). The keratinous filaments in the outer shell membrane are more numerous and thicker than in the inner membrane. Their arrangement is largely parallel to the shell, whereas in the inner membrane filaments are intertwined, running in all directions. Wolken & Schwartz (1948) observed by electron microscopy that the dried shell membrane of the hen egg consisted of freely intertwining fibres 1 µm thick, with about 20 x 10^6 equal-sized pores per cm^2. Fluids and gases pass through the shell membrane by diffusion.

The shell membranes and yolk membrane are similar in chemical composition (Table 5), but their physical structure differs (Wolken, 1951).

The amino-acid composition of the inner and outer membranes is similar, but the former has more proline, the latter more arginine and cystine. Sodium, potassium, magnesium, iron, manganese, copper, aluminium and phosphorus were present in almost equal amounts in the two membranes and in the cuticle. There was less calcium in the inner shell membrane. Bauman (1968), in his monograph indicated that in view of its chemical composition, the protein in egg-shell membranes could not be keratin, which contains more cystine, nor collagen, which contains oxyproline, lacking in shell membrane. Because of the high content of aspartic and glutamic acid, he believed that the protein might be related to the collagenous protein of cartilage, especially as both proteins had been found to contain hexosamines. Vadehra et al. (1971) studied the physico-chemical properties of egg-shell membranes and confirmed that membrane proteins were different from keratins, and that the name ovokeratin was thus inappropriate.

Cooke & Balch (1970) established a correlation between the weight of the inner and outer shell membranes ($r = 0.79$). Both membranes contain the same organic substances although in different concentrations. A significant correlation between the thickness of cuticle and shell membranes was found by El-Boushy et al. (1968).

Table 5. Composition of the shell cuticle and membranes, expressed as mass fractions (w) (? of total solids). Data from Maesso et al. (1969).

	Cuticle	Membranes	
		outer	inner
Water-insoluble protein	0.80	0.92	0.85
Total carbohydrates	.	0.04	0.015
Lipids	0.10	0.02-0.04	0.02-0.04

It is commonly assumed that greater thickness of shell membranes impairs gas exchange of the embryo towards the end of incubation, which in turn reduces hatchability. The data of Cooper (1970) to some extent contradict this assumption. In his experiments, hatchability of turkey eggs did not depend on shell-membrane thickness in eggs from laying hens of one breed, but it did vary between different breeds.

The chemical composition of the hen's egg-shell varies over a considerable range (%): $CaCO_3$ 94.9-97.9; $MgCO_3$ 1.3-1.8; $Ca_3(PO_4)_2$ 0.8; P_2O_5 0.4-0.8; K 0.3; organic substances 4.1-5.5 (Romanoff & Romanoff, 1949). Calcium carbonate in the outer layer of shell has the form of crystals arranged along the longitudinal axis, at right angles to the shell boundary, while in the inner layer it is amorphous.

Nady & Olah (1956) in a spectroscopic study of trace elements in hen egg-shells, demonstrated that shell material contained the following mass fractions of elements: (mg/kg) Fe 4-7, Zn 0.5-2, Cu 0.2-1; (µg/kg) Ag 50-500, Mg 50-1000, Ti 100-500, Pb 50-600, Ni 50-500, Cd 20-500, Sn 0-600, Co 0-100, Mn 0-30.

The organic basis of the shell, according to Bauman (1968), makes up about 5% of its weight and consists of proteinaceous fibrils including a large amount of acid mucopolysaccharides. The mineral part of the shell is composed almost entirely of calcium carbonate in the form of calcite, with minor admixtures of magnesium and calcium phosphates. In his opinion, alkaline phosphatase participated in deposition of the organic basis of the shell, and carbonic anhydrase was involved in the phosphorylation of its minerals. His data showed that carbonic anhydrase was three times as active in the uterus as in other parts of the hen's oviduct. Apparently this enzyme does not catalyse the hydrolysis, but the synthesis of carbonic acid with subsequent dissociation of H_2CO_2 into H^+ and HCO_3^- (the bicarbonate ion). Support for this hypothesis is provided by the acid reaction in uterine cells (pH = 5.8), at which the carbonate ion cannot be formed.

During incubation, shell mass diminishes by 11.5% of its initial value, the amount of ash in the shell by 8.9% and organic matter by 21% (Shpits, 1966). The embryo thus draws almost half (46.9%) of all ash elements from the shell.

The quality of the egg shell is influenced by feeding, housing, and physiological factors (state of the laying hen's nervous system, diseases, etc.). Mongin (1968) reviewed data on the role of blood acid-base balance of the laying hen in the physiology of egg-shell formation. He concluded that calcium was the primary factor in determining shell formation, and CO_3^{2-} second.

The shell must be sufficiently firm to support the sitting hen, and yet thin enough for the chick to break out without effort. There is a direct correlation between volume of the chicken egg (40 - 60 cm^3) and shell thickness (0.34 to 0.39 mm) (Romanoff & Romanoff, 1949). The shell is thickest at the sharp end; in winter, it is thicker than in summer. El-Boushy et al. (1968) found a significant correlation between the individual shell layers and total thickness of the shell. Climatic stress (high temperature and humidity) had a considerable effect on the laying hen during

egg formation and caused a significant reduction in shell tickness. A statistically significant positive correlation was established between shell thickness and mass density of eggs (Mountney & Vanderzant, 1957). According to Svensson (1957), there is a relationship between mass density and hatchability of eggs. The highest percentage hatched from eggs with an average mass density of 1.075-1.080 kg/litre. Payne & McDaniel (1958) obtained somewhat different results; with increasing mass density of turkey eggs (i.e. the thicker the shell), the number of eggs not hatched decreased. This matter was studied in detail by Shpits et al. (1965). They divided eggs into 5 groups: (1) mass density 1.0675 kg/litre, and shell thickness 0.285 mm; (2) 1.0735 and 0.31; (3) 1.0795 and 0.326; (4) 1.0855 and 0.35; (5) 1.0925 and 0.380. In groups 1 and 2 abnormalities of shell and internal structure of the egg were most frequent, and when incubated they had the highest percentage of infertile eggs (22.3-19.2% against 13.3-15.7% in the other groups), and embryos dying in the first week of incubation (9.2-7.2% against 4.7-5.1% in the other groups). Shpits & Danilova (1966) believed that thicker shells were associated with higher biological value of eggs, as reflected by better use by the embryo of egg nutrients, by higher embryo mass, greater hatchability, better quality of chicks both at hatching and during subsequent development, and greater productivity of the adult hen. They noted a tendency for inheritance of egg-shell quality.

The number of pores, their size and distribution in the shell vary widely both between flocks and among chickens in the same flock (30 - 170 pores per cm^2). But they vary little in eggs from the same hen. According to Lomova (1939), eggs from Langshan hens have fewest shell pores (85/cm^2) and those from Bantam the highest (149/cm^2). The average on White Leghorn eggs was 127 per cm^2. She points out that the number of countable pores diminishes with increasing staining intensity of the egg shell. There is little correlation, if any, between shell thickness and pore number. This was confirmed by Svensson (1957). According to Otrygan'ev (1966), eggs from hens of the laying breed Russian White have 113 ± 5.2 pores per cm^2 shell, against 94.3 ± 3.7 for the broiler breed Cornish, which would, according to him, ensure a smaller mass loss during storage for 18 days (2.36% compared to 3.85% for Russian White).

Pores are not evenly distributed over the surface. At the blunt end, they averaged 151, in the middle 142 and at the sharp end 100/cm^2 (Vladimirova, 1954). The pore opening is usually oval. The pore size is to some extent related to egg size, but it varies widely even on a single egg. The pore canal narrows inwards, where it ramifies into a network of air canals in the prismatic layer of the shell. Rauch (1952), using a new method, studied shell porosity of eggs from which chicks had hatched, and in which embryos had died during incubation (Table 6).

He found that most eggs with dead embryos had larger pores, resulting in excessive permeability of the shell and excessive evaporation, which killed the embryo. Part of the eggs with dead embryos, however, had shells with pores of normal diameter, so that some deaths must have had another cause.

Table 6. Diameter (d) of shell pores and its statistical parameters, number of pores (N) divided by surface area (A) and pore area ($A(p)$) per shell from eggs that have hatched and that contained dead embryos. Data from Rauch (1952).

	d/µm			$100s(d)/\bar{x}(d)$	$N \cdot A^{-1}$/cm^2	$A(p)$/mm^2
	$\bar{x} \pm s$	min.	max.			
Hatched	9.4 ± 0.5	1.6	25.8	57.4	148	0.7
Dead	21.8 ± 1.4	1.6	74.7	64.7	101	2.5

The shell surface may be smooth or rough, glossy or dull. The latter two properties are determined by a greater or smaller amount of cuticle. Rough eggs often appear at the end of the laying season. Kuchkovskaya (1938) reported that the proportion that hatched of eggs with rough shells was lower (51.4-68.2%) than of those with smooth shells (85.8%).

The cuticle of the hen's egg is 0.005-0.01 mm thick. Simons & Wiertz (1966) established that the cuticular surface of the hen's egg was porous and that the pores are filled with air. The cuticle consisted mainly of mucin, was fairly stable in structure and dissolved only when eggs are dipped into hot water (over 40 °C). The cuticle contained much glycine, aspartic acid and glutamic acid, but little proline, methionine and tyrosine (Maesso et al., 1969). The same authors found more calcium in the cuticle than in the shell membranes, but equal amounts of sodium, potassium, magnesium, iron, manganese, copper, aluminium and phosphorus. The cuticle also contained fat droplets.

The role of the cuticle in permeability of the shell to gases is differently interpreted. Marshall (1947) observed that, when the shell cuticle was destroyed, evaporation from eggs did not increase, but decreased. He assumed that shell permeability to moisture was not related to pore surface (number of pores multiplied by mean diameter), but to the surface of crater spots in the cuticle, at the bottom of which are the relatively small pore holes. Pores stained in a relatively dry atmosphere looked smaller, while at 80-90% humidity they were larger, creating optimum conditions for evaporation. He suggested that cuticle spots play a role like that of leaf stomata in regulating the release of moisture from the egg. But according to data of Walden et al. (1956), removal of the cuticle and shell membranes increased permeability of the shell to gases.

1.2. Fertilization of the ovum; embryo development before laying; role of yolk and white in embryo development

The egg is fertilized in the infundibulum, and initial development of the embryo occurs during passage through the oviduct, when white, shell membranes and shell are deposited around the yolk (about 24 h). Embryo development starts 3 h after fertilization, with division of the ovum into 2 cells. These cells in turn divide by a second cleavage, at right angles to the first.

As the avian ovum is overfilled with yolk, division takes place only at the animal pole, where the cytoplasm contains a small amount of yolk granules. Alongside and underneath the blastodisc some cells remain incompletely separated from the yolk. The nuclei of some cells, as well as those of excess sperm cells, participate in reorganization of the yolk to facilitate its assimilation by the embryo. Bekhtina (1960), who studied development of the fertilized ovum, established that the multicellular blastodisc is formed when the egg is in the uterus, its outer layer having the appearance of epithelium, while underlying cells are more loosely arranged. The peripheral zone of the blastodisc consists of large incompletely separated segments of protoplasm with many nuclei (the yolk syncytium). This zone is not divided from the underlying yolk and is called the germinal disc. Merocytes liquify part of the yolk underneath the blastodisc, thereby forming a cavity filled with fluid, the subgerminal cavity. The fluid is formed by transudation of moisture through the blastoderm from the white above it. This was established experimentally by New (1956), by culture in vitro of a blastodisc under a layer of white. The subgerminal fluid provides a better medium than yolk for enzymic processes essential for embryo nutrition at this early stage of development, and for the dispersion of breakdown products excreted by the embryo. Van Deth (1962) presented evidence that the subgerminal fluid was formed by active resorption of water and nutrient solution from protein of the living tissue of the blastoderm and not by digestion of the yolk. A different opinion was held by Sheinis (1964) who believed that the yolk latebra formed a cavity containing fluid which exudes from the yolk into the subgerminal cavity.

The part of the blastodisc above the subgerminal cavity is transparent, and is called the zona pellucida. As the blastodisc increases in size, it moves toward the yolk surface beyond the germinal disc, arranging itself directly onto the yolk. This part of the blastodisc is opaque, and is called the zona opaca.

At the time of oviposition, the blastodisc is a small whitish spot on the yolk surface, with an average diameter of 4.4 mm in fertilized eggs and 3-4 mm in unfertilized eggs (Romanoff & Romanoff, 1949). According to some investigators, the blastodisc in fertilized eggs has reached the gastrula stage at oviposition, but Knorre (1959) regards this as the early gastrula stage.

Eggs in which embryo development has not started after setting in the incubator are not always infertile. Munro & Kosin (1945) examined histologically freshly laid eggs and showed that up to 75% of eggs regarded as infertile, died during the first 25 h of development. Thus already inside the oviduct, there is a period of increased embryo mortality.

When laid, the egg shows considerable variation in embryo development, depending on how long it stays in the oviduct. Observations indicate that if the egg stays longer in the uterus, the interruption in embryonic development at laying may reduce hatchability. Zusman (1968) found in laid chicken eggs blastodiscs of varying size and stage of differentiation: (1) more developed blastodiscs with fully formed entoderm and a greater number of

cells in the core, although smaller than in blastodiscs of less advanced stages of development; (2) normal; (3) retarded. He noted a positive correlation between blastodisc size and embryo development during the first two days of incubation, the rate of development of blastodiscs being equal at all stages. In autumn a larger proportion of blastodiscs was observed with more development, and in spring the proportion with retarded development was higher. Coleman & Siegel (1966) found in freshly laid eggs from a line of hens laying light eggs 72% blastodiscs in the gastrula stage and 28% in the pre-gastrula stage, whereas in lines laying heavier eggs these percentages were 27 and 73, respectively. But they observed no great differences in blastodisc size with either weight of chicken lines or stage of differentiation. Arora & Matsumoto (1968) investigated blastodiscs of unincubated eggs in three weight groups (1: 65-70 g; 2: 57-62 g; 3: 49-54 g), and reported that blastodisc size was proportional to eggweight, while degenerating blastodiscs were more frequent in the larger eggs; greatest variation was observed in the latter after 38 h incubation, and subsequently greatest mortality: three times more than in medium-sized and small eggs.

Vladimirova & Sergeeva (1968) made the interesting observation that the pH of white and yolk differs in eggs with fertile and infertile blastodiscs. It was 5.85 for yolk and 8.66 for white in fertile eggs and 5.9 and 8.69, respectively, in infertile eggs. The authors ascribed this to accumulation of carbon dioxide in fertile eggs by metabolic processes.

Zlochevskaya (1965) demonstrated that blastodisc size at oviposition is a heritable trait. Blastodiscs in eggs from laying hens raised intensively from eggs, became earlier visible at ovoscopy than when the hens stemmed from eggs with slower development. Subsequent embryo development was closely related to the initial stage; chicks in the first group had more erythrocytes, whereas those in the second group had more leucocytes indicating weakness of chicks with slow development. Working with Cornish and Russian White hens, Orlov & Zlochevskaya (1966) confirmed the results of their previous research which showed that a higher proportion of eggs with early embryo development hatched. Dolbeneva & Orlov (1966) demonstrated that an advance in embryo development, as established by candling at less than 18 h incubation, was maintained in the post-embryonic period until the age of 45 days; at 75 days this difference in chickens disappeared (Coleman & Siegel, 1966).

Iordanov (1969) believed that in science there was insufficient appreciation of the complex interrelations between the embryo and other parts of the egg, of which it forms an integral part. The reason for this was that for a long time scientists confined their interest to the embryo.

Morphological investigations revealed that early in development, intracellular yolk was utilized, and was taken up by the blastodisc during cell division. The yolk is rapidly consumed, and during the ensuing incubation the peripheral yolk provided a direct medium for the blastodisc. This yolk is easily assimilated, as shown by both histological and histochemical parameters, and

by some data about its effect on the blastodisc. The yolk underneath the blastodisc with its intact globules forms a barrier to expansion of the blastodisc border, but the differentiated endoderm overcomes this barrier, assimilating the yolk globules by a special mechanism intermediate between phagocytosis and pinocytosis. Thus, the surface layers of the yolk are also involved in early embryonic metabolism. According to Iordanov (1969), the view that the latebral yolk is the first nutrient source for the embryo (Needham, 1931) is not justified. Accordingly, the assumption of Sheinis (1964) that the embryo receives its first food from the latebral cavity is also inaccurate. Iordanov further reports that the latebral yolk is utilized comparatively late by the embryo, after the peripheral and surface yellow yolk. The latebra has long ago been attributed the role of keeping the yolk in position within the egg with the blastodisc on top an important condition for gas exchange of the early embryo. Indeed, the form and position of the latebra are of decisive importance for this, but to explain this mechanism solely on the basis of lower mass density of the latebral yolk is untenable cytologically.

A necessary substrate for early development of the blastodisc is the yolk membrane. The blastodisc has been experimentally explanted onto yolk membranes, mostly synthetic. Essential conditions were the structure of the inner surface of the yolk membrane, facilitating attachment and migration of ectodermal cells from the blastodisc border, along with the extreme permeability of the membrane both for small molecules and macromolecules.

The presence in yolk of globules is important for prolonged maintenance in the laid egg of a disequilibrium in the concentration of ions diffusing between yolk and white, and concomitant differences in pH and osmotic pressure between them before incubation. The main barrier preventing equilibration between yolk and white is not the yolk membrane, but the boundary between peripheral and yellow yolk. The globules of yellow yolk, which are in close contact with one other and have a semi-permeable surface layer, form a compact barrier (parallel to the yolk surface and mutually alternating), preventing free diffusion of substances from outside. During embryo development, this barrier is broken by the destruction of macro- and microstructures in the yolk by the growing blastodisc, allowing also penetration of water and ions essential for embryo development, from the white into the subgerminal cavity and from there to the embryo.

The existence in yolk of separate microstructures (yolk globules) and their phagocytosis by cells of the blastodisc early in development seems to ensure the most effective assimilation of a highly concentrated food reserve, that forms the essence of both globules and yolk granules. A number of immunological, serological and morphologic data suggest that phagocytosis is the first step in the transfer from yolk to the embryo of some readily available proteins and lipids in unchanged form.

Analysis of the nutritional value of yolk and white and their individual substances and fractions early in development is based mainly on data from explanation in vitro. An easily diffusing yolk fraction of low molecular weight, supplemented with a small

quantity of serum allows normal culture of some tissues, which may be attributed to the set essential amino acids and vitamins of the B group present in this fraction. The livetin fraction of yolk is absorbed by cells of the blastodisc for protein synthesis. Egg white supports and sometimes stimulates cells grown in vitro, but it cannot be directly absorbed for protein synthesis.

The yolk and white are in equal measure the main energy sources in early morphogenesis and growth of the embryo. Some studies indicate that bound carbohydrates of the livetin fraction from yolk can also provide energy after enzymic splitting of the glycoproteins in that fraction. Proteins of yolk, but not of white, are nitrogen sources for protein synthesis by the embryo at early stages of development. All protein fractions of yolk are equally involved in metabolism early in embryo development. This agrees with data indicating that yolk globules are phagocytosed by the blastodisc, and confirms that the yolk globule is a highly specialized functional structure in the ovum.

The livetin fraction supplies the major proteins of the intercellular substance (plasma) for embryonic blood. Free amino acids do not appear to be utilized by the embryo for protein synthesis, but have another, hitherto unknown function. The DNA pool in yolk is utilized as a source of precursors for nuclear DNA, which is synthesized early in embryogenesis. Brachet (1961) assumed that a close relation exists between DNA synthesis and the production of energy in the egg.

Egg white participates in embryo metabolism from an early stage of development. Besides supplying water and creating for the embryo a medium with particular (alkaline) pH, osmotic pressure, and ratio of sodium to potassium, egg white provides the embryo with glucose, the main nutrient for morphogenesis (together with yolk), and with low-molecular compounds which stimulate complete utilization of the yolk reserve. Varnagiris (1967) takes a different view. After removing 8-9 ml white from duck eggs after 72-74 h incubation, he found that embryos had the same weight after 15 days, but after 24 days incubation their weight was significantly less than controls. He concluded that during the first 15 days white was not utilized for embryo nutrition. It should be remarked, however, that white is, in fact, consumed in the first three days of incubation, earlier than the start of his experiment.

Iordanov concluded that an inseparable functional unity exists between the embryo, early in development, and its main food components, yolk and white.

1.3. Factors influencing hatchability

Tolokonnikova (1964) showed experimentally that hatchability [proportion of eggs hatching] was influenced by the weight ratio of white to yolk, more than by any other biochemical factor. When this ratio was from 1.81 to 2.02, hatchability was 82.4-88.2%, but below 1.80 or above 2.03 hatchability dropped to 57.4-69.2%. A lesser factor was the protein index: at 0.068, hatchability was 77.95%, and at protein indexes of 0,048 and 0.092, values were 74.09 and 60.49%, respectively. Moiseeva & Tolokonnikova (1968)

later established that the following factors exert a significant influence on hatchability (in decreasing order): shell thickness, fertility of eggs, solids in white and yolk, relative weight of white, relative amount of inner thin white, protein index, intensity of lay, and relative amount of thick white; the content of cholesterol and of vitamins B_1 and B_2 had no effect on hatchability, but this finding was reversed in a subsequent study (Kushner et al., 1971).

We shall examine in greater detail the shape and weight of eggs as related to hatchability. As shown by Rogulska & Komar (1969), orientation of the embryo in the egg and its concordance with von Baer's law depend on egg shape: the rounder the egg, the greater is the deviation from this law. This causes disturbances in embryo development, incorrect orientation before hatching and, thus, a decrease in hatchability. Confirmation was provided by Schneider (1969) who found more unhatched among round eggs. Vladimirova & Sergeeva (1969) measured a large number of eggs and established that the shape index [ratio of length to diameter] of the normal egg of laying hens is 1.36 and of broiler chickens 1.34, and that sharp divergences from the normal shape decrease hatchability. Karapetyan & Arutyunyan (1966) recommended incubation of eggs from broiler-mother hens with a minimum shape index of 1.38, minimum index of thick white 0.08, minimum yolk index 0.438, and a minimum shell thickness of 323 µg.

Contradictory statements exist on the influence of egg weight on hatchability. Most present investigators conclude that the highest hatchability is found with average egg weight for a given breed, line or flock. We shall cite some recent studies on this matter.

Eremeev (1969) confirmed earlier reports that higher egg weight is associated with higher weight of chicks, weight of yolk sac, dry weight of bones and ash. He noted, however, an inverse correlation between egg weight and weight of chick relative to egg before incubation, i.e. heavier eggs yield chicks with relatively less weight. Noting a similar variability in egg weight (by coefficient of variation) for hens of different flocks, and in various species of bird, he regarded this as aromorphosis ensuring biological progress for the avian class. Vladimirova & Sergeeva (1968) emphasized that the correlation between egg weight and chick weight is particularly high for the first batch of chicks taken from the incubator (i.e. for the shortest incubation periods): 0.88 against 0.35 for the second batch. In another study (1967), they established a high positive correlation (r = 0.42 to 0.99) of egg weight and weight of white, yolk and shell with length and transverse diameter of the egg, and also between egg weight and shell thickness; between the refractive index of white and its content of solids and the height of white in Haugh units. They further established that the highest hatchability from eggs from Russian White hens is observed with following parameters: mass density 1.090-1.095, ratio of white to yolk 1.80-2.14, Haugh units 60-70, refractive index of white 1.3540-1.3470, pH of white 8.6-9.0, pH of yolk 5.8-6.1. Deviations from these values lowered hatchability. Eggs from Plymouth Rock hens had slightly different

limits, but the best hatchability was observed in eggs with average values for all quantities.

According to data of Marion et al. (1964), eggs from three lines of hen genetically differing in egg weight, showed statistically significant differences in the mass fraction of white, yolk and shell in whole egg, and of solids in white. For each gram increase in egg weight, mass fraction of white increased by 0.2% and of yolk decreased a like amount. They assumed that genetic variation in egg components is only a covariation with egg weight. Kazachkina (1970) found that in eggs of average weight the refracture index of white was higher (and so was hatchability) than in small and large eggs. She also observed that protein content and refractive index of white was higher in eggs laid in the morning than in those laid in the evening. Of particular interest was her observation that highly productive hens laid eggs whose white was more alkaline [? had a higher pH]. Litko (1968) obtained the highest hatchability of Plymounth Rock eggs weighing 59-61 g (82.4% of eggs set), and for eggs of the same weight from Cornish x Plymouth Rock crosses hatchability was 83.9. In the next weight range (62-64 g), there was a slight drop in hatchability only in crosses (to 82.6%), while in crosses of other weight groups, and in eggs of all weight groups from Plymouth Rock hatchability was strongly reduced (to 10-30%). Nyland (1969) observed highest hatchability (85-92.5%) from eggs weighing 55.5-58.5 g. For eggs weighing 50-53 g hatchability was 80%, and for the 59.5-65 g weight group only 74%. According to Tsarenko & Tsarenko (1970), best hatchability was observed in eggs weighing 56-60 g (from Cornish hens 88%), with egg weights below 56 g and above 60 g 7 and 12% less [81 and 76%].

An opposite conclusion was reached by Schneider (1969), who found that in White Leghorn and White Rock hens the higher the weight and mass density of eggs, the lower the proportion of unfertilized eggs and dead embryos. A particularly sharp drop in hatchability accompanied a mass density less than 1.070 kg.litre^{-1}. Similar data were obtained by Bocharova & Sopikov (1970). Eggs with an average weight of 69.0 $\pm$ 0.6 g from Leningrad White hens had a considerably higher hatchability (85%) than those weighing 61.1 $\pm$ 0.4 g (76.2%). Pel'ttser (1966) preferred incubation of larger eggs for the following reasons: egg weight had a heritability coefficient of 60-75%; chicks hatched from large eggs had a higher liveweight. Incubation of large eggs thus selects towards large strains. He believed that egg weight could be increased by selection for 2-3 generations.

Favret et al. (1964) found that selection by embryo mortality had a greater effect in reducing variability in weight of eggs that hatched, than on the decrease in mean weight of all fertilized eggs. Natural selection thus plays a stabilizing role, eliminating extremes. They assumed that on this basis an optimum phenotype could be established. Landauer (1967) believed that the relationships between egg weight and hatchability were extremely complex. Some factors had an immediate influence, such as the interaction between surface area of the egg and rate of dehydration, and the proportion of white and yolk, varying with egg size. Other factors

genetic and environmental, influence egg size and so have an indirect effect on hatchability.

Many studies have dealt with the effect of age of the laying hen on egg quality. Recent data are cited here.

With increasing age of laying hens, Zlochevskaya (1969) observed higher weight and mass density of eggs, higher mass fraction weight of yolk and shell in egg, of solids in white and yolk, in particular the amount of sugar. The latter plays an important part in embryo development, especially during the first days of incubation. Bolton (1957) found that eggs from hens aged 4-5 years had more porous shells and lost one and a half [?2½] times as much weight as those from pullets, both in the incubator and at room temperature.

Because of their smaller mass fraction of shell weight and larger fraction of yolk, the eggs of old hens had a higher specific enthalpy of combustion Table 7).

Table 7. Effect of age of hen on egg weight and physical composition, and on energy value (specific enthalpy of combustion. *m*, mass; *w*, mass fraction; *h*, specific enthalpy of combustion.

Age (years)	*m*(egg) (g)	*w*(of whole egg)/g·kg^{-1}		*w*(of white)g·kg^{-1}			*h* (kJ·kg^{-1})
		shell	yolk	thick album.	thin album.	thick albumen	
6-10	47.9	82	363	393	162	708	7.70
1- 2	55.6	88	321	311	280	526	7.06
1	37.6	104	297	311	311	481	6.68

Marion et al. (1964) observed that eggs from three lines of hens in the second year of production contained 0.7% less shell and 0.5% more yolk [? had a mass fraction of shell 0.007 less and of yolk 0.005 more]. In another study (1966), they obtained statistically significant differences in egg weight, mass fractions of parts and chemical components, both in different lines and in the same laying hens, at differing ages. The age of the laying hen largely determined mass fraction of moisture, lipids, fat-free solids and of most fatty acids in the egg. High correlations were noted between mass fractions of yolk, white, moisture, lipids, and of individual fatty acids. In eggs from laying hens of one flock, there was a negative correlation between egg weight and mass fraction of yolk; on the other hand, with increasing age the increase in egg size was accompanied by an increase in mass fraction of yolk. They concluded that the greater sizes and constant correlations between mass fraction of yolk and age of laying hen point to a major effect of age of laying hen on the amount of yolk.

According to data of Kondratenko (1970), the mass fraction of white decreased, and its mass increased with increasing age of the laying hen while the mass fraction of solids remained constant: in other words, water accounted for the increase in mass of white, thus reducing the protein index [? its mass fraction of

protein] and quality of white. The mass of yolk increased with age through an increase in solids, chiefly fat. As the height of white increased with age of the laying hen, Munro (1971) assumed that pH of white would go up too. But the contrary proved true: the pH of white dropped by 0.08-0.15 with increasing age of the laying hen.

According to Pigarev et al. (1966), the hatchability of eggs laid by pullets aged 6-7 months was not more than 80%; hatchability increased as hens grew older, and at 12 months 88% of fertilized eggs hatched. They recommended selection for incubation of eggs weighing at least 50 g from pullets of up to 8 months, and 53 g or more from older hens. Bessarabov & Grigor'eva (1966) confirmed that hens aged 12-13 months laid heavier eggs (53.8-65.9 g) of better quality (greater mass of thick white and smaller mass fraction of shell) thans hens of 8-9 months (mass of egg 50.1-58.2 g). The better quality of eggs was also apparent in greater hatchability: 82.4-84.6% against 74.6-78.8% for hens of 8-9 months.

Anorova (1956) observed that embryo development in eggs from pullets was less advanced (early gastrula stage) than in those of hens in the second year of lay (late gastrula stage). She believed that these differences were responsible for the more advanced development, seen in the first few days of incubation, of embryos in eggs from older hens. Breslavets (1967) noted several changes in egg quality with increasing age of laying hens: higher mass of egg through increased mass of yolk, lower refractive index of the outer thick, and inner thin white; greater number of somites after incubation for 1½-2½ days, apparently associated with a more advanced stage of the blastodisc at oviposition and a higher rate of growth; 1.1-3.0% increase in hatchability in various breeds. Dogadaev (1968) confirmed these data.

According to Pel'ttser (1969), the offspring obtained from sexually mature parents that had completed growth would be more viable, and would have a higher and more stable productivity than offspring from pullets. In the first days of incubation, embryos in eggs from older hens had a higher mass than those in pullet eggs and this difference persisted throughout incubation.

Buvanendran (1968) reached opposite conclusions from data on the influence of parental age on fertility and hatchability of Rhode Island eggs (Table 8).

Table 8. Effect of parental age on fertilization and hatchability. Data from Buvanendran (1968).

	Fertilization (%)	Hatchability (%)
Cockerel x pullet	89.5	82.3
Yearling x pullet	88.8	83.2
Cockerel x yearling	84.0	81.0
Yearling x yearling	74.3	77.2

Hatchability diminished with age of the laying hen; age of the male played a positive role when they are mated with pullets (hatchability increased by 0.9%), but a negative role when mated with year-old hens.

We shall examine in greater detail the heritability of hatchability. Moiseeva (1961) studied the heritability and variability of particular features in eggs laid by dams and daughters. She found highly significant correlations in mass of egg (0.50), of egg contents (0.51), and of white (0.58). She assumed that poor heritability of any feature might be due to certain factors, like environmental conditions (as for hatchability); or selection for a given feature. The value of a particular property is vital for the animal, and through natural selection an average value is reached (mass of yolk, mass fraction of solids in yolk), so that if the animal requires a certain value for a property, the parameters of its variability, and the heritability coefficient would be slight. In a survey of genetical literature on hatchability of birds' eggs, Knize & Rydlová (1965) concluded that embryo viability depended both on egg quality and on a complex of properties determined by the genotype of both parents. Breed or line characteristics of eggs are more subject to maternal influence, as manifest in hatchability in reciprocal matings: if the dam belonged to a breed with higher hatchability than that of the sire, 1.9-14.2% (mean 7.3%) more eggs hatched than with the reciprocal mating. According to data of Ivanova (1970), eggs from hens with a high laying rate (0.6 d^{-1} or more) had high hatchability. The correlation between laying rate and hatchability was significant (r = 0.239-0.359).

Hassan & Nordskog (1967) considered that the genetic basis for duration of incubation did not depend on mass of egg. They presented data, however, showing that eggs of average mass had the shortest incubation and that incubation of heavier eggs lasted 24 h longer on average. Nagai & Gowe (1969) established that coefficients of genetic correlation between quality parameters of eggs from one laying hen (mass and mass density, height of white and Haugh units) were very high: 0.91-0.96 within a laying season, and 0.76-0.87 between laying seasons. They concluded that to define egg quality for purposes of breeding it is sufficient to analyse 2-3 eggs per pullet during any two laying cycles up to 10 months of age.

It is well known that inbreeding for selection and reinforcement of certain useful features adversely affects hatchability. Dogadaev (1968) found that eggs from inbred hens weighed 1.1 g less than from outbred hens; their yolks weighed less and represented a smaller fraction of the egg; they had a greater mass fraction of white, which was of poorer quality; and hatchability was reduced: 76.9% of eggs laid and 88.4% of those fertilized against 87.0 and 91.8% for eggs from outbred hens. Embryo development was slower in eggs from inbred hens: after incubation for 18 h, the proportion of eggs with visible embryos reached 80.6% against 90.5% for eggs from outbred hens.

Moiseeva (1970) studied the effect of a high inbreeding rate (70%) on egg quality. She found that hatchability dropped from

92 to 84.4%; mass of egg from 53.3 to 49.3 g; mass of white from 33.3 to 28.0 g; mass of yolk and mass fraction of solids remained unchanged. The coefficient of variation for 10 properties out of 16 studied was higher in inbred chickens. The phenotypic and genetic variability in inbred chickens of the examined flock, despite a high inbreeding rate, was attributed to the absence of special selection for the features studied. Silin & Kikavskii (1969) reported a 4.1-9.5% drop in hatchability for brother x sister matings in Cornish and Plymouth Rock fowl, but no effect on hatchability was observed when half-brothers were mated with half-sisters. They recommended the latter type of mating for reinforcement characteristics being selected. According to Bernier et al. (1951), the relation between inbreeding and hatchability is not linear since mortality of embryos was greatest at initial stages of development.

Kushner et al. (1971) mated New Hampshire hens with Russian White cocks and found hybrid vigour in mass of egg, mass fraction of yolk, mass and mass fraction of thick white in egg, of solids and protein in white, and a decrease in mass fraction of cholesterol in yolk lipids, from 50.52 to 47.35 g/kg. Shtele (1970) mated White Leghorn fowl of different lines which were alike in egg morphology, chemical composition and pH of white and yolk. Hybrid vigour of embryos was displayed in growth of the vascular system and of the embryo itself, and in better utilization of white and yolk. This resulted in 6-8% increase in hatchability over the parent lines.

In an interesting study, Tolokonnikova et al. (1970) found that several egg properties were interrelated as well as correlated with hatchability. Geretic correlations between properties frequently did not coincide with phenotypic ones, as fairly high correlations existed between these and the environment (for example, dependence of egg quality on nutrition and on storage conditions) (r = 0.13 - 0.51).

Hays (1959) reported results of an attempt to breed, over a period of 11 years, different lines of Rhode Island Reds with 'high' and 'low' hatchability. He drew the conclusion that the concept of 'hatchability' is an abstraction whose physiological basis is too complex to warrant its use as in selection.

Moiseeva (1965) investigated variability of eggs within the daily laying cycle, and reported that the first egg was usually laid early in the morning, the second by 10:00, the third at noon, and the last at 13:00-16:00; between the first and last egg, mean mass decrement of egg was 1.54 g, of contents 1.14 g, of white 1.25 g, of shell 0.16 g, and mass fraction decrement of solids in white by 0.25%. The mass of yolk and mass fraction of yolk solids were more stable. Hatchability was highly variable, and depended more on environmental factors. Pingel & Bock (1969) also studied changes in properties of eggs within a laying season. They found that the last egg weighed more than the earlier ones, height of thick white increased from egg to egg while shell thickness decreased in the last eggs, especially in pullet eggs at the onset of lay through lack of calcium.

Merat & Lacassagne (1961) observed that hatchability from one-

egg batches was lower than from batches of 7 eggs and more, but within a batch hatchability was about the same. On the other hand, Bernier et al. (1951) found that eggs laid in the middle of a cycle were better hatchable than the first and last.

Besides genetic factors, egg quality and thus hatchability, are influenced by environmental conditions, such as feeding, housing and season.

The effects of feeding and housing of the laying hen on egg quality have been fairly well investigated. One study will be mentioned here. Intensive housing of hens and cocks on a wire mesh floor, compared to extensively housed flocks kept in small poultry pens with runs, altered the mass fraction of fatty acids in yolk and improved hatchability (Jones, 1968).

Let us deal in greater detail with seasonal influences on egg quality. According to data of Karapetyan & Arutyunyan (1966), mass of egg and of white was more and of yolk less in autumn/winter; in spring, mass of shell weight was greater; and in summer with high temperatures, mass of eggs, white and shell was less but of yolk more and the mass fraction of thick white, yolk and shell was less. Bessarabov & Grigor'eva (1966) found that in summer hen eggs contain more carotenoids, and in autumn more fat in yolk and more ash in white. Tarabrina et al. (1968) established a dependence of mass and some other properties of hen eggs on season and temperature. Egg mass rose until April (temperature 2-5 ^{o}C) to 59.8 g on average. With falling temperatures in September, egg mass again started to rise. The lower mass during summer was accounted for by a lower mass of white, but the amount of thick white decreased more rapidly. In summer, egg shells were more porous than in winter.

Anorova (1956) noted that in autumn eggs stay longer in the oviduct than in summer and, accordingly, the blastodiscs were more developed in eggs laid in autumn. Lutz & Lutz-Ostertag (1957) found seasonal differences in embryo development in freshly laid duck eggs. It was more advanced in eggs laid in spring and was equal to that in winter eggs incubated for a few hours.

1.4. Measuring incubation properties of eggs

Interesting work by Vladimirova & Sergeeva (1969a; 1971) is available on ways of measuring incubational properties.

We present here only a few data on general requirements for incubation eggs and a short description of new methods of measuring incubational properties of hen eggs.

The selection of eggs for incubation has two aspects: quality assessment of batches of eggs to find how far eggs from a particular breeding flock meet requirements for incubation; and the actual sorting of eggs for incubation.

One of the external criteria used for egg evaluation is shell colouring. Godfrey (1950) established that New Hampshire eggs with dark-brown shell, high mass density and little dehydration during incubation for 14 days were better hatchable than eggs of lighter tint. Pingel (1958) claimed that the superior hatchbility of dark-tinted eggs was due to a denser shell, which limited de-

hydration. Drugociu et al. (1956) found that Rhode Island eggs with heavier pigmentation had a more regular shape (ratio of length to diameter 1.30-1.41), higher hatchability (87.3% against 54% for weakly pigmented eggs), and yield more viable chicks. Vladimirova & Sergeeva (1969c) found a positive correlation of 0.3, between shell colour of Plymouth Rock broiler eggs and the colour of yolk. Light cream eggs had 64.3% hatchability (of fertilized eggs); cream eggs 75.7%, dark cream 82.3%, but brown eggs had 79.5% fertilized eggs.

The vitamin content of incubated eggs plays an important role in hatchability. Maslieva (1950) established a direct relationship between the content of vitamin A in yolk and hatchability; mass fraction of vitamin A in yolk could therefore serve as a criterion for assessment of incubation properties. When the yolk contained 32.3 µg vitamin A, hatchability was 93.8% of fertilized eggs. A lower mass fraction of vitamin A corresponded to lower hatching rate, and when it was 13.3 µg, only 69.3% eggs hatched. Eremeev (1956) observed a positive correlation between the content of carotenoid pigments, which are provitamins A, in egg yolk and shell thickness. As noted by Pel'ttser (1969), hatchability of eggs with brightly coloured yolks, containing large amounts of carotenoids, was 7-8% higher of pale eggs. Vladimirova & Sergeeva (1969b) established that there is a correlation between yolk colour and its content of carotenoids which is independent of breed, age of laying hen and season. They proposed a scale defining amount of carotenoids in egg yolk.

We cite a number of general recommendations about incubated eggs. Vladimirova & Sergeeva (1968) recommended selection of eggs for incubation by the following parameters: mass density 1.082-1.085 kg/litre; shape index 1.34-1.36; pH of white 8.6-8.7; and pH of yolk 5.7-5.9. Hatchability was markedly lower when mass density was 1.070 kg/litre or less (Schneider, 1969). Summarizing a vast amount of data, Moiseeva & Tolokonnikova (1968) presented optimum ranges of egg properties related to hatching. The data were obtained empirically and have been compiled theoretically according to formulas proposed by the authors (Table 9).

Incubation properties may also be assessed by parameters of embryo viability. Orlov (1954) recommended the use of blastodisc size in selecting eggs for incubation, instead of indirect criteria (external properties of the egg). He presented extensive evidence that after warming of eggs for 12 h at 37.5-40.5 °C, hatchability (in %) of those with well developed blastodiscs was 11.5 higher (3.4 through warming and cooling and 7.9 from elimination of unfertilized eggs and eggs with poorly developed embryos). Bukhovets (1966) repeated Orlov's experiment and proposed that, rather than physical characteristics which do not reflect physiological processes, embryo viability should be adopted as criterion for egg evaluation. After incubation for 16-18 h, eggs were sorted into two groups according to size [diameter] of blastodiscs: 1) blastodiscs of up to 3.5 mm; 2) 4-8 mm. The eggs in Group 1 yielded 60% chicks, those in Group 2 85-90% or more, and viability of chicks was also 3% higher. Blastodisc size was not related to egg weight, but the weight of eggs and chicks did correlate.

Table 9. Limits of some egg properties for hatchability from empirical data and calculated from theoretical formulae. Data from Moiseeva & Tolokonnikova (1968).

Property	Limits	
	empirical	theoretical
Egg weight/g	46-55	49.7-58.7
Shell thickness/mm	0.36-0.44	0.37-0.43
Index of white	0.07-0.09	0.07-0.11
w(thick albumen)/10^{-3}	460-600	455-573.5
w(inner thin white)/10^{-3}	220-280	227.9-291.9
w(outer thin white)/10^{-3}	120-220	145.8-249.3
Haugh value	74-82	74.5-89.5
m(white)/m(yolk)	1.80-2.40	1.96-2.32
w(solids in white)/10^{-3}	126-142	122.3-137.5
w(solids in yolk)/10^{-3}	524-540	530.7-544.3
w(lipids in yolk)/10^{-3}	347-362	351.9-366.9

In our opinion, this method of assessing eggs for incubation by embryo viability is highly sophisticated.

We cite some new methods for evaluation of egg quality. One such method was proposed by Bekhtina & Dyagileva (1968). They found that fluorescence of the egg shell, a feature specific for the laying hen, to some extent reflects her physiological condition, which in turn influences egg quality. Shell fluorescence has the following peculiarities: it changes with seasons (diminishing from February to June, then increasing again); it decreases with age of the laying hen; it is significantly correlated with shell thickness ($r = 0.353 \pm 0.13$); it is correlated with hatchability. It has also been found that the proportion of female chicks declines with increasing fluorescence: in Russian White $r = 0.21 \pm 0.08$ at $P = 0.95$, in New Hampshire $r = 0.34 \pm 0.11$ at $P = 0.99$. New Hampshire eggs with shell fluorescence 65-86 yielded 61.1% female and 39.9% male chicks but when fluorescence was 153-176, the proportions were 20 and 80%, respectively. In our opinion a simplified method for estimating fluorescence would make this test suitable for use on breeding farms. A number of methods for evaluation of incubation eggs were proposed by Limarenko (1968). He considered that existing methods of egg selection were unsuitable through lack of instruments permitting objective measurement of properties proposed for selection. Tsarenko (1969a) suggested a new method for assessment of incubation quality. He candled eggs and measured the intensity of light that passed through the egg (through its small diameter), using a photoelectric element and a sensitive galvanometer. The following data were obtained: approximately 99% of the light passed through the egg was absorbed, most of it by the shell and shell membranes (about 50%) and the yolk (about 44%); the shell of a freshly laid egg lets through about twice as much light as one laid 12 hours earlier, and 3 and 5.5% less after 36 and 60 hours; thereafter light absorption remains constant; the greater diffusion of light

through the shell of fresh eggs is due to the content of moisture in its pores; eggs with the highest fraction of light diffusing through have the highest hatchability; when it decreases, hatchability declines by 19% of the eggs set. He concluded that fraction of light diffusing summarizes internal egg quality, and may serve as a selection feature for egg incubation. Kazachkina (1970) introduced a new method by analysing 0.5 ml white extracted before incubation. She established a significant correlation between mass fraction of protein content of white and the refractive index, and another correlation between mass fraction of protein in white and hatchability ($r = 0.685 \pm 0.04$). When refractive index of white was in the range 1.3580-1.3609, hatchability was 95% of fertilized eggs and 84.8% of set eggs; with 1.3550-1.3570, it was 79 and 69%; with 1.3520-1.3549, 70.5 and 61.2%; with 1.3490-1.3519, 78.5 and 55.5%, respectively. Mention should also be made of an interesting method of monitoring embryo development in the first days of incubation, which was proposed by Turevskii et al. (1967) and which involves vital staining of embryos with neutral red.

With the advance of poultry farming, egg quality is improving and sorting before incubation less indispensable. With extensive data, Fomin (1950) showed that if the maternal flock were in good condition and properly fed, the preliminary sorting of eggs for large-scale incubation was pointless, since it increased hatchability by less than 1%. By supervising the laying performance of individual hens, those producing eggs with poor hatchability (60% and less) could be rejected. Their removal raised hatchability by 10%. Shpits et al. (1965) recommended periodic estimation of mass density of eggs for every laying hen, which should be culled if mean mass density was 1.0675-1.0735 kg/litre; such eggs frequently have defects in the shell and inner structure and a poor hatching rate.

We believe therefore that on organized poultry farms, it is now most efficient to check incubation properties of eggs of every laying hen periodically instead of laboriously sorting all eggs before incubation.

2. The egg during storage

During egg storage, two main types of changes should be considered: changes in the condition of the embryo, and in its environment (the egg's contents).

2.1. Changes in the egg's contents during storage

The first sign of an effect of storage time is enlargement of the air cell due to evaporation of water from the egg. According to data of Becker et al. (1968), the hen egg lost on average 0.04 g per 24 h during storage at temperature 11.7-13.9 °C and relative humidity 75%. Vladimirova (1954) related weight losses of eggs (entirely due to moisture loss) to profound and irreversible physico-chemical changes in the egg's contents, causing deterioration of incubation properties, in particular reduced weight of white, disturbance of structure (after 10 days storage), decreased electrical conductivity and a rise in pH of white and yolk, especially in the first 5 days of storage. A different opinion was held by Mueller (1959), who found a very low correlation (r = 0.21) between Haugh value and loss of water during storage. To compensate for moisture loss during storage, Bartold (1957) proposed that stored eggs be soaked in warm water (38 °C) to swell the contents, expel gases dissolved in white and yolk, and air from the air cell, and then transferred to cold water (15-18 °C), causing them to suck water. He claimed that this procedure would render the eggs fresh again. Steinke (1966) checked this method, but did not find significantly greater hatchability. Kaufman (1939) also denied that embryo mortality increased after, extended egg storage by moisture loss, because fractional decrease in mass after 34 days storage was 0.7-1.5%, whereas a decrease of 3-6% by artificial reduction of pressure did not increase embryo mortality during incubation. Consequently dehydration of eggs during storage is not the main cause of decreased hatchability.

In addition to evaporation of water from the egg, the transfer of water from white to yolk occurs during storage by difference in osmotic pressure. The extent of this transfer is related to a change in thickness and elasticity of the yolk membrane. According to Brooks & Taylor (1955) a daily average of 0.05 g water passes from white to yolk during storage at 0 °C, and twice as much at 10 °C, which sharply reduces the viscosity of yolk. Viscosity relative for water is for yolk with a water content of 47.2% 100, but for yolk with 57.6% water 3.5. The passage of water from white to yolk during storage causes marked distension of the yolk membrane and alters the shape of the yolk, sometimes rupturing the yolk membrane. Vladimirova & Sergeeva (1968) regarded yolk

index (ratio of height to diameter) as the main criterion of egg freshness. In Russian White eggs, for example, the ratio of height to diameter of yolk dropped from 0.46 (in fresh eggs) to 0.36 during storage for 15 days. The yolk membrane may also burst by a change in its quality during egg storage. Britton (1971) showed that the mass fraction of solids in the yolk membrane had decreased after storage for 14 days at 12 °C, and particularly at 22 °C.

During storage the white liquefies characteristically by disturbance of the structural coherence and makes the yolk highly mobile, so that it floats towards the shell. The blastodisc, situated on the yolk, thereby touches the shell membrane and suffers mechanical damage. Mueller (1959) believed that liquefaction of white was largely due to transfer of water from white to yolk, with a highly significant correlation of 0.594. A correlation of 0.815 was established between the Haugh value of egg white, before and after storage. He demonstrated too that all symptoms indicating deterioration of egg quality during storage were interrelated. In an earlier paper, Mueller (1956) advanced the idea that the negative correlation between evaporation of water from stored eggs and Haugh value depended on a positive correlation between evaporation of water and release of carbon dioxide from the egg. In his opinion, the limited solubility of CO_2 in water explained the higher Haugh value of egg white and the higher content of CO_2, in eggs stored in saturated air.

Sauveur (1967) did not believe that a correlation existed between reduced hatchability and the decrease in Haugh value during storage. During storage, carbon dioxide is lost by release of gas dissolved in the egg's contents or by breakdown of carbonic acid. According to Sauveur (1967), the fresh egg contained about 55 mg CO_2. Release of CO_2 raised the pH of white. He reported that dissociation of carbonic acid in the egg depended on the CO_2 content of the store room. He devised the following formula:

$$\text{pH of egg} = 6.37 + \lg \frac{1.125\rho(HCO_3)}{0.03p(CO_2)} - 0.12$$

where 6.37 is the dissociation constant of H_2CO_3; $p(CO_2)$ is the partial pressure of CO_2 in the atmosphere; 0.12 is the activity coefficient of HCO_3; $\rho(HCO_3)$ is the mass concentration of bicarbonates in g per litre white.

Release of carbon dioxide from the egg's contents raises the alkalinity of both white and yolk: the pH of white from 7.6 to 9.7 and of yolk from 6.0 to 6.8 (Shkeir, 1970).

Cotterill et al. (1958) confirmed the earlier observation that loss of CO_2 from eggs is accelerated by increased temperature, and thus the solubility of CO_2 in white is inversely proportional to an increase of temperature. The adverse effect of high storage temperature on egg quality was strongly retarded by storage in carbon doxide (Cotterill & Gardner, 1957).

According to Limarenko (1968a), after 25 days storage the pH of white had risen from 8.7 to 9.8, of yolk from 6.3 to 6.5. The temperature, ranging from 4 °C to 10-15 °C in that period, did not influence the pH change of white and yolk. Potokina & Protsenko (1970) found that during storage the pH of yolk rose from

6.06 to 6.09; the refractive index of yolk dropped from 1.4193 to 1.4183.

The reader should not be confused by the differences in pH values, because the investigators used eggs from hens of different flocks and breeds. Moreover, experiments were done in various seasons, and with different temperatures and storage times. Important are the direction and sequence of the changes, which is why data obtained by different investigators have been presented.

The effect of pH on the interaction between lysozyme and ovomucin was studied in vitro to elucidate the mechanism of liquefaction of white during storage according to Cotterill & Winter (1955). An increase in pH reduced the intensity of this interaction. At pH 9-9.5, it disappeared and the white started to liquefy. They supposed that the interaction between lysozyme and ovomucin to some extent facilitates the maintenance of thick white in the gel state. The absence or decrease of this interaction would cause the thick white to liquefy. Baliga et al. (1971) demonstrated that before storage the concentration of ovomucin in solid white was four times as high as in thin white. This difference had not changed after 30 days storage, but the thick white contained a third as much ovomucin as the initial concentration. The reduction in ovomucin accompanying liquefaction did not result in release of free hexosomes and hexosamines. No change was observed in the amount of free glucose, the only sugar remaining after liquefaction of thick white.

From their finding that 24 h storage at 37.5 ^{o}C removes all carbon dioxide from white, Becker et al. (1968) concluded that for increased embryo viability it was important to raise the percentage of carbon dioxide in the air during storage, rather than during incubation.

Swartwood & Spencer (1967) found that a CO_2 concentration 0.01-1.5% during storage delayed degradation of white (expressed in Haugh units), but had no marked effect on the speed of liquefaction of white. A.Y.M. Smith (1931) found that an increase in pH of white was prevented by the following storage conditions: at 0 ^{o}C the CO_2 concentration should be 2-3%; at 20 ^{o}C 3-4.5%; at 38 ^{o}C 5-7%. Sauveur et al. (1967) reported that broiler eggs had 4% CO_2 after laying, and only 2% after 24 h storage under normal conditions. They observed that in eggs stored for three weeks in 4% CO_2 the pH of white was 7.5, but with 2% CO_2 it was 8.46. They regarded pH 8.2 as optimum for white. In experiments by Shkedov (1971) enrichment with carbon dioxide before incubation yielded a greater increase in hatchability when initiated on the 7th day of storage, than after 10 days storage. It follows that release of CO_2 from eggs cannot be the only cause of embryo mortality in eggs stored longer.

A high positive correlation exists between the release of CO_2 and water vapour from eggs during storage, while the correlation between CO_2 release and permeability of shell to bacteria is negative but scarcely significant (Reinke & Baker, 1966).

Antonenko et al. (1965) compared various methods of storage (one hour warming per day; 5 hours warming every 5 days; 5 hours warming on the first day; and no warming as control) and reached

the following conclusions: The storage method did not affect vitamin A and carotenoid content in yolk. Even storage for 25 days did not cause a loss of these substances. With every storage method tested, the pH of white rose to an equal extent, and was related only to the duration of storage. The lysozyme titre was strongly affected by the method of storage; in the control it dropped 10^{10} times. The smallest decrease was noted with 5 hours warming every fifth day, 2×10^4, with 5 hours warming on the first day the titre reduction amounted to 5×10^6 and with daily warming for 1 hour it was 10^7. The earlier quoted paper by Cotterill & Winter (1955) should be recalled, which discussed the relation between ovomucin and lysozyme, and the reduced quality of thick white when this relation was disturbed. The drop in lysozyme titre may well be the principal cause of 'ageing' of eggs during storage.

The data of Snyder (1957), and Bornstein & Lipstein (1962) indicate that the decrease in Haugh value during storage is not related to age of laying hen. But given the fact that the initial quality of eggs depends on the age of the laying hen, and the highly significant correlation between the decrease in Haugh value and initial egg quality (Mueller, 1959), there seems to be a relation between the reduction in Haugh value and age of the laying hen.

Carter (1969) found a positive correlation of shell deformative stress with relative humidity during storage, and a negative correlation with storage time. He assumed that reduced deformative stress was caused by a change in elastic properties of the organic shell component. Tolokonnikova et al. (1970) reported a sharply decreased correlation between mass density and firmness of the egg shell in the course of storage.

Limarenko (196 a) presented data on changes in shell fluorescence, size of air space and other egg parameters during storage (Table 10).

Sauveur (1967) repeatedly noted the absence of basic studies on the effect of individual storage conditions on internal characteristics, which could clarify how these conditions affect hatchability.

2.2. Changes in the blastodisc

The most interesting question is what happens to the embryo during storage, when it approaches an anabiotic state.

Orlov (1948) studied the effect of methods of egg storage on embryo viability, and assessed the state of blastodiscs in eggs that had been classed as unfertilized after 6 days incubation. Out of 45 'unfertilized' eggs stored for 15 days before setting, 36 proved to be fertilized on opening, but their blastodiscs were at the development stage of freshly laid eggs. Of 90 eggs believed to be unfertilized after 25 days storage, 70 were fertilized.

Kaufman (1938) studied the biological effect of storage on embryonic development. Hen eggs were stored at 12 °C for 24, 28 and 34 days. Control eggs from the same hens of the same weight were set one day after laying in the incubator. It was noted

Table 10. Changes in shell fluorescence, size of air space and index (diameter divided by length) during storage. Data from Limarenko (1968a).

Time stored (days)	Egg fluorescence	Air space			Diam./length	
		diam. (mm)	height (mm)	dehydration (g)	albumen	yolk
		At 4 °C				
1	bright red, crimson	up to 6	up to 1.5	none	0.078	0.470
2-4	same (somewhat weaker tone)	10.0	2.6	0.14	0.072	0.440
5-7	red (slightly weaker tone)	12.5	3.4	0.272	0.071	0.425
8-10	red	13.7	4.6	0.420	0.070	0.405
11-15	pink	15.1	4.8	0.776	0.060	0.372
20-25	pink (weakly purple tone)	up to 16	up to 5	up to 1.43	0.055	0.350
		At 10-14 °C				
1	bright red, crimson	up to 6	up to 1.5	none	0.078	0.047*
2	red (slightly weaker tone)	12	3.2	0.42	0.068	0.435
5-7	red (strongly weaker tone)	17	4.0	1.48	0.065	0.385
8-10	pink	20.5	5.7	2.05	0.054	0.361
11-15	weakly purple	21.5	6.5	2.44	0.056	0.327
20-25	same (appearance of blue spots)	up to 23	up to 7	up to 5.22	up to 0.06	0.305

* Error: perhaps 0.470

It was noted that after prolonged storage, mortality was sharply increased in the first week of incubation. The relative water content of embryos was higher, and their weight at the age of 7 and 14 days was considerably less than of the controls. The relative growth, however, was more advanced in the third week of incubation. This supports our finding of a biological compensation pattern (Rol'nik, 1968). When the metablism of embryos had slowed down at the start of incubation through some circumstance, increased metabolic activity was observed subsequently, compensating as it were for the delay.

Kaufman (1939a,b) noted a 24-hour increase in incubation time over a control, for eggs stored longer (34 days). This was not due to slower growth of the embryo, but to a later initiation of development (also by about one day). We present data of Funk (1934) in increased incubation times after prolonged storage (Table 11), which were later confirmed by Kaufman (1938) and Zawalski (1962).

Table 11. Effect of storage time on duration of incubation. Data from Funk (1934).

Trials	Storage time (days)	Required incubation time (h)	
		range	mean
1	1-7	492-528	512
	8-14	498-546	522
	15-17	516-546	530
2	1-7	498-540	513
	8-14	504-540	518
	15-21	510-540	527

MacLaury & Insko (1968) established that storage time has a twice as much influence on duration of incubation as egg weight. Crittenden & Bohren (1961) calculated that every storage day increases the incubation time by 0.7 h, and according to Becker et al. (1968), by about 1 h. Such a conclusion does not seem warranted, as deterioration of egg quality, and the resulting extension of incubation time, should be differentiated by periods; the beginning of egg storage (Day 1-10); Day 11-20; Day 21-30.

Neel (1942) found a correlation of 0.77 between rate of embryonic development and hatchability. Bohren et al. (1961) showed that a connexion existed between reduced hatchability and extended incubation time, thus confirming Olsen's data (1951) that those eggs had better hatchability which had been less affected by storage at 0 °C.

According to Ivanov (1955), storage of hen eggs for 6-19 days not only lowered hatchability (from 98.9 to 75.4% of fertilized eggs) and prolonged incubation time (from 20 to 21.5 days), but also shifted the sex ratio: 63.3% pullets from fresh eggs and 41-47% from stored eggs.

Sittmann et al. (1971a) observed that temporal changes in zygote viability during storage were similar in three bird species: fowl, quail and turkey. With extended storage time, they noted a sharp rise in the proportion of dead blastodiscs on the first day of incubation, that is before vascularization. Extended storage time of eggs from inbred chickens caused an even greater drop in hatchability. Hatchability of eggs from inbred hens stored for less than 10 days amounted to 85% of that of outbred hens, with 11-20 days storage it was 53%, with 21-30 days 41%, and when the storage period exceeded 31 days 22% (taking the hatchability of eggs from outbred hens as 100% for the same period).

Shishkina (1949) presented data on the development of 36-h chick embryos after various storage periods (Table 12).

She noted that tissue differentiation was strongly retarded in embryos from eggs that had been stored for long periods. Embryos could have 2-3 pairs of somites when the edges of the neural tube had not yet grown together. Like Orlov (1948), Shishkina believed that different conditions should be applied in the first days of incubation for eggs stored for various periods.

Table 12. Development of 36-h embryo during storage. Data from Shishkina (1949).

Time stored (days)	Number of embryos examined	Mean length of embryo (mm)	Mean diameter of vascular field (mm)	Ratio of length of embryo to length of vascular field	Mean number of somites	Mean number of somite pairs per mm embryo length
0	23	5.76	7.2	1:1.38	9.88	1.88
5	21	5.00	7.0	1:1.42	7.57	1.51
10	21	4.88	6.6	1:1.00	6.53	1.34
15	21	4.14	6.4	1:1.54	6.60	1.59
20	17	3.47	5.0	1:1.44	2.40	0.69

Weisbroth & Kosin (1966) observed a change in the outer shape of blastodiscs in turkey eggs after a 14-day storage period: widening at some places and narrowing at others. They pointed out that differences in embryo development between fresh and stored eggs were noticeable throughout the incubation period, but were particularly pronounced at early stages. In another study (Arora & Kosin, 1968a) structural details gradually disappeared and numerous vacuoles appeared in the light and dark field of the blastodisc. They suggested that the presence of vacuoles was a symptom of tissue degradation, large vacuoles precluding embryo development.

Pel'ttser (1966) remarked that the longer the storage and the worse the conditions of storage, the more the embryo was retarded in growth and development. Since this retardation at initial stages of development could not be fully compensated afterwards, maximum attention must be given to proper conditions for egg storage.

Bykhovets (1966) found that after 5-7 days of storage, the growth of blastodiscs during incubation was slower, but if eggs had been warmed daily for 2 h during storage, their blastodiscs developed like those in fresh eggs. From a study of blastodiscs in eggs stored for long, Steinke (1966) concluded that changes resulting from storage without warming were irreversible. No damage occurred in blastodiscs of eggs stored with short warming periods. Sittmann et al. (1971b) found that a delay in the onset of embryo development after prolonged storage increased the number of morphological abnormalities, especially eye and beak defects, as well as the number of twins (Table 13).

According to data of Arora & Kosin (168b), the resistance to egg storage differed in two genetically isolated lines of broad-breasted bronze turkeys (one with high, the other with low hatchability). In eggs of the high-hatchability line, blastodiscs showed less abnormalities even after 21 days storage, and there were less necrotic and fragmentary cells.

Zusman (1969) found that during storage at optimum temperature (6-8 ^{o}C) some life processes continued in the blastodiscs of hen

Table 13. Incidence of abnormalities (especially eye and beak defects) and of twins (percentages of eggs). Data from Sittmann et al. (1971b).

Time stored	Days			
	2-10	11-20	21-30	31-40
Eye or beak defects				
outbred	3.3	6.6	4.9	11.2
inbred	3.2	6.4	4.3	19.8
Twins				
outbred	0.7	1.0	0.5	2.2
inbred	1.1	1.2	0.9	4.7

eggs. In particular, an increase in volume of nuclei and accumulation of deoxyriborucleate was observed, which varied in blastodiscs of different size and stage of differentiation. The delay in development was reversible. At suboptimal storage temperatures or when storage was extended beyond 3-4 days, a deviation occurred in the distribution of stages of embryo development. In fresh eggs, 18-27% embryos were observed with retarded development; in eggs stored for 3 days at 8 °C, 30-32%; in those stored for 5 days at the same temperature, 50%. The best embryonic development was seen in eggs stored for 3 days at 22 °C. At this temperature development had begun but had not yet become disorganized, so that after 48 hours incubation the percentage of embryos with normal and advanced development was even a bit higher than in those set in the incubator directly after laying. He supposed that changes in the proportion of various groups of embryos (retarded, normal and advanced) during storage was due to a varied reaction of blastodiscs which, at oviposition, were at different stages of development (from pregastrula to late gastrula).

A few words should be said here about the effect of embryonic stage at ovoposition on speed of embryo development and on hatchability. If embryo development was more advanced at laying, incubation time was reduced.

Kosin (1956, 1964) reported that the varying degrees of development of embryos at oviposition were largely responsible for differences in their viability.

Sturkie & Williams (1945) confirmed this with the following refined experiment. Eggs were removed from the uterus a few hours before oviposition, with blastodiscs at an earlier stage of development. Hatchability was very slow, and in some eggs, development did not even start.

Coleman & Siegel (1966) also reported different reactions to the duration of storage, in embryos in varying stages of development at oviposition. Low-weight eggs, whose blastodiscs at oviposition were mainly at the gastrula stage, were not affected by storage for 14 days under favourable conditions (78% hatchability), while heavy eggs with blastodiscs mainly at the pregastrula stage, required 4 hour warming at 37.5 °C during storage, to raise hatchability from 74.2% (storage without warming) to 78.4%. Kosin (1956),

by warming eggs during storage, induced a defined stage of embryonic development and thereby raised hatchability. If, however, embryonic development was optimum at oviposition, warming not only failed to increase hatchability, but could even lower it. Sauveur (1967) supposed that this difference in embryonic stage at oviposition could in fact be an explanation for the conflicting data obtained by various workers with warming of eggs during storage.

Kaufman (1938) found that during storage catalase activity was decreased in white, and increased in yolk. His data indicate, however, that the activity of this enzyme was in no way related to embryo mortality.

Injection into eggs of small amounts of glutathione for 2 weeks of egg storage (within a total storage time of 30 days) decreased embryo mortality, and consequently increased the hatchability. He assumed that disturbance of the redox system was one of the harmful effects of storage. Enzyme systems were very sensitive to changes in pH and other physico-chemical conditions. We therefore believe that disturbance of enzyme activity is a major cause of reduced embryo viability during extended storage. Unfortunately, little work has been done on changes in different enzyme systems during storage.

We shall briefly survey the effect of prolonged storage on development of chicks after hatching. Kaufman & Krzanowska (1957) found that after storage of hen eggs for 16-21 days at 10 ^{o}C the total weight of hatched chicks was reduced, as was the weight of the intestinal tract and the liver. Activity of the thyroid and utilization of haemoglobin by the liver was higher in embryos from stored eggs than in controls, which suggests more intensive metabolism. Lorkiewiczowa (1960-1962) observed a weight decrease in chicks hatched from eggs stored for more than 10 days; chick mortality was raised when the eggs had been stored for over three weeks.

If, during storage, the shell cuticle was partly destroyed (worn off), embryo viability was severely impaired. According to Vadehra & Baker (1968), eggs without cuticle (removed from the uterus) were contaminated with microflora on the 5th day of storage to the same extent as normal eggs after 20 days storage. The protection afforded by the cuticle did not change appreciably during 96 h storage. Differences are found only in some lines and breeds of fowl.

Irradiation with ^{60}Co of hen eggs before a 15 day storage period, at doses of 1-20 r [rad], raised hatchability by 12.1-13.7%, the hatchability in the control being 74.5% of fertilized eggs. Also increased was the percentage of healthy chicks, by 6.9-9.1%. Unfortunately, Shkeir (1970) does not explain the beneficial influence of irradiation, because changes in pH were similar in irradiated eggs and control, and only vitamin B_2 was better maintained in the experimental groups.

For comparison, we cite data of some workers on the effect of storage time on embryonic development in other poultry species. Vladimirova (1962) noted that in goose eggs the size of blastodiscs was reduced after prolonged storage, and their appearance

became curbly. Shevtsova (1965) studied the effect on embryo development of storing duck eggs for 7-14 days, and established that the processes of embryonic development were disorganized; strong inhibition of growth was observed on days 2-4 incubation and reduced rates of cell division, as a result of which on day 8 of incubation the embryo contained almost 2.5 times less nitrogen than in freshly laid eggs. The author believed that storage did not cause irreversible changes in embryonic enzyme systems because the ability to grow was retained, but it did increase embryo mortality and reduce hatchability. Tsarenko (1969a) ascribed the better ability of duck eggs to survive extended storage, than of hen eggs, to their greater weight. The best hatchability for duck eggs was observed after 6-11 days storage, and for hen eggs after 2-3 days. He assumed that during storage a 'ripening' process took place, similar to the ripening of potato tubers. He did not, however, indicate what would be the biological essence of such a ripening, nor did he clarify the accompanying processes within the egg. This hypothesis conflicts with known data and we join the majority of investigators in their belief that eggs should be set in the incubator as soon as possible after oviposition and should be stored only when absolutely necessary.

References

1. Anorova, N.S., 1956. Features of the early stages of embryonic development in eggs from chickens of different age. Dokl. Akad. Nauk SSSR 110:494 (in Russian).
2. Antonenko, N.V., N.V. Volokitina, T.D. Kolupaeva & L.L. Shornikova, 1965. Biochemical parameters during egg storage. Ptitsevodstvo 5:27 (in Russian).
3. Arora, K.L. & L.L. Kosin, 1968a. The response of the early chicken embryo to preincubation temperature as evidenced from its gross morphology and mitotic pattern. Physiol. Zool. 41(1):104.
4. Arora, K.L. & I.L. Kosin, 1968b. A heritable difference in the resistance of turkey blastoderms to prolonged preincubation storage - cytological evidence. Poultry Sci. 47:2000.
5. Arora, K.L. & L.H. Matsumoto, 1968. Early embryogenesis in chicken eggs of different weights. Proc. Iowa Acad. Sci. 75:405.
6. Baliga, B.R., S.B. Kadkol & N.L. Lahiry, 1971. Thinning of thick albumen in shell eggs; changes in ovomucin. Poultry Sci. 50:446.
7. Ball, H.P.Jr. & O.J. Cotterill, 1971. Egg white catalase. 1. Catalytic reaction. 2. Active component. Poultry Sci. 50:435; 446.
8. Barkovskaya, O.V., 1954. Determination of the speed of yolk formation in chicken eggs. Fiziol. Zh. SSSR 40:722 (in Russian).
9. Bartold, 1957. Cited by Steinke (164).
10. Bauman, V.K., 1968. Kal'tsii i fosfor. Obmen i regulyatsiya u ptits. (Calcium and phosphorus. Metabolism and regulation in birds.) Riga (in Russian).
11. Becker, W.A., J.V. Spencer & J.L. Swartwood, 1968. Carbon dioxide during storage of chicken and turkey hatching eggs. Poultry Sci. 47:251.
12. Bekhtina, V.G., 1960. Early stages of cleavage in the chicken embryo. Arkh. Anat. Gistol. Embriol. 38:77 (in Russian).
13. Bekhtina, V.G. & G.E. Dvagileva, 1968. Egg fluorescence and economic features of chickens. Nauchn. Tr. Pushkinskogo nauchn.-issled. Lab. Razved. sel'sk.-khoz. Zhivotn. 2(12):154 (in Russian).
14. Bellairs, R., M. Harkness & R.D. Harkness, 1963. The vitelline membrane of hen's egg: a chemical and electron microscopical study. J. Ultrastr. Res. 8:339.
15. Bernier, P.E., L.W. Taylor & C.A. Gunns, 1951. The relative effects of inbreeding and outbreeding on reproduction in the domestic fowl. Hilgardia 20:529.
16. Bessarabov, V.A. & N.A. Grigor'eva, 1966. Some age and season-

al changes in the quality of pullet eggs. In: Mater. VI vses. nauchno-metod. Konf. Zoogigiene Osnovam Veterinarii: 154 (in Russian).
17. Bocharova, I.G. & P.M. Sopikov, 1970. Characteristics of the embryonic development of Leningradskaya Belaya chickens as related to egg weight. Mater. II Konf. molodykh Uchenukh Genetike Razvedeniyu sel'sk.-khoz. Zhivotn. VNII Razvedeniya Genetiki sel'sk.-khoz. Zhivotn. 1:184 (in Russian).
18. Bohren, B.B., L.B. Crittenden & R.T. King, 1961. Hatching time and hatchability in the fowl. Poultry Sci. 40:620.
19. Bolotnikov, A.M., A.I. Shurakov, Yu.N. Kamenskii & V.K. Korolev, 1970. Conditions and nature of the development of bird embryos at the time of oviposition. Ekologiya 4:40.
20. Bolton, W., 1957. The effect of age of the hen on the porosity of the egg. J. agric. Sci. 49:384.
21. Bornstein, W. & B. Lipstein, 1962. Some characteristics of measures employed for determining the interior quality of chicken eggs. Br. Poultry Sci. 3:127.
22. Brachet, J., 1961. Biokhimicheskaya embriologiya. Moscow. (presumably Russian edition of: The biochemistry of development: New York, 1960).
23. Breslavets, V.A., 1967. Effect of chicken age on the quality of incubation eggs, and on the embryonic and post-embryonic development of the offspring. Sbornik Rabot molodykh Uchenykh VNITIP 9:301 (in Russian).
24. Britton, W.M., 1971. Chemical composition of the vitelline membrane of mottled and non-mottled yolks. Poultry Sci. 50: 1558.
25. Brooks, J. & D.J. Taylor, 1955. Eggs and egg products. Food Invest.spec. Rep. No. 60.
26. Buvanendran, V., 1968. Influence of age of parents on fertility and hatchability in the Rhode Island red. Poultry Sci. 47:636.
27. Bykhovets, A.U., 1966. Selection of eggs for incubation raises viability of the bird. Trudy UNII Ptitsevodstva: 32 (in Russian).
28. Carter, T.C., 1969. The hen's egg: variation in shell deformation under static load with relative humidity and age of egg. Br. Poultry Sci. 10:311.
29. Chung, R.A. & W.J. Stadelman, 1961. The physical and chemical components of the hen's egg as related to total egg weight. Poultry Sci. 40:1387.
30. Coleman, J.W. & P.B. Siegel, 1966. Selection for body weight at eight weeks of age. 5. Embryonic stage at oviposition and its relationship to hatchability. Poultry Sci. 45:1008.
31. Cooke, A.S. & D.A. Balch, 1970. Studies of membrane, mamillary core and cuticle of the hen egg shell. Br. Poultry Sci. 11: 345.
32. Cooper, J.B., 1970. Thickness of turkey egg shell membranes and hatchability. Poultry Sci. 49:1376.
33. Cotterill, O.J. & F.A. Gardner, 1957. Retarding thick white deterioration by holding shell eggs in sealed containers. Poultry Sci. 36:196.
34. Cotterill, O.J., F.A. Gardner, E.M. Funk & F.E. Cunningham,

1958. Relationship between temperature and carbon dioxide from shell eggs. Poultry Sci. 37:479.
35. Cotterill, O.J. & A.R. Winter, 1954. Egg white lysozyme. 1. Relative lysozyme activity in fresh eggs having low and high interior quality. Poultry Sci. 33:607.
36. Cotterill, O.J. & A.R. Winter, 1955. Egg white lysozyme. 3. The effect of pH on the lysozyme-ovomucin interaction. Poultry Sci. 34:679.
37. Crittenden, L.B. & B.B. Bohren, 1961. The genetic and environmental effects of hatching time, egg weight and holding time on hatchability. Poultry Sci. 40:1736.
38. Deth, J.H.M.G. van, 1962. Changes in water, sodium and potassium distribution in the duck eggs during incubation. Austr. J. exp. Biol. Med. 40:173.
39. Dogadaev, A.M., 1968. A comparative investigation of the quality of eggs, hatchability and viability in inbred and outbred chickens. Sb. Rab. molodykh Uch. VNITIP 10:9 (in Russian).
40. Dolbeneva, E.F. & M.V. Orlov, 1966. Viability and growth of chicks as related to intensity of metabolism during incubation. Trudy VNITIP 30:96 (in Russian).
41. Drugociu, Ch., Al. Dascalu, N. Neaga, I. Pamfilie, O. Turliuc & V. Petrus, 1956. Study of some factors affecting hatchability of Rhode Island eggs. Buletin sti. Acad. Repub. pop. rom. Fil. Iasi, Sect. Biol. sti. Agric. 7:209 (in Rumanian).
42. El-Boushy, A.R., P.C.M. Simons & G. Wiertz, 1968. Structure and ultrastructure of the hen's egg shell as influenced by environmental temperature, humidity and vitamin C additions. Poultry Sci. 47:456.
43. Eremeev, G.P., 1956. Dehydration of eggs with different carotene levels during incubation and storage. Ptitsevodstvo 2:39 (in Russian).
44. Eremeev, G.P., 1969. Some interrelationships between egg weight and the processes of growth and embryonic development in chickens. Nauchn. Trudy Omsk. sel'sk.-khoz. Inst. 71:32 (in Russian).
45. Favret, E.A., E. Lifschitz & F. Manso, 1964. Cited by Landaur (
46. Ferdinandov, V.V., 1931. Working manual on poultry farming. Moscow, Sel'khozgiz (in Russian).
47. Fitzsimmons, R.C. & P.E. Waibel, 1968. Free amino acid composition of the avian egg as related to embryonic development. Poultry Sci. 47:219.
48. Fleming, A. & V.D. Allison, 1924. On the antibacterial activity of egg-white. Lancet 1:1303.
49. Fomin, A.I., 1950. Effectiveness of various methods of egg selection for incubation. Trudy pushkin. nauchno-issled. Lab. Razv. sel'.-khoz. Zhivot. 4:88 (in Russian).
50. Funk, E.M., 1934. Factors influencing hatchability in the domestic fowl. Missouri agric. Exp. St. Res. Bull. 341.
51. Godfrey, G.F., 1950. On the nature of genetical control of egg shell quality as it affects hatchability in domestic fowl which lay brown eggs. Ohio State Univ. Abstr. doct. Diss. 60 (1948-1949)131.

52. Greenwood, A.W. & W. Bolton, 1956. Variations in the amounts of thick and thin albumen in hens eggs with the age of the bird. J. agric. Sci. 47:40.
53. Hassan, G.M. & A.W. Nordskog, 1967. Influence of selection for body weight and egg weight on embryonic growth rate and hatching time. Poultry Sci. 46:1271.
54. Hays, F.A., 1959. Breeding for high egg production. Massachusetts agric. Exp. St. Bull. 504.
55. Ivanov, V.G., 1955. Influence of egg storage time on sex ratio in the offspring of chickens. Dokl. Akad. Nauk SSSR 100: 1021 (in Russian).
56. Ivanova, I.B., 1970. Analysis of parameters of hatchability in a population of Plymouth White chickens. Mater. II Konf. molodykh Uchenykh Genetike Razvedeniyu sel'sk.-khoz Zhivotn. 1:180 (in Russian).
57. Il'in, M.D., 1917. Study of embryonic development in the chicken egg. Materialy dlya Embryokhimii. Petrograd (in Russian).
58. Iordanov, Zh.S., 1969. The cytobiology of the chicken egg. Publ. Bulg. Acad. Sci. (in Bulgarian).
59. Jolles, J., J. Hermann, B. Niemann & P. Jolles, 1967. Differences between chemical structures of duck and hen egg-white lysozymes. Eur. J. Biochem. 1:344.
60. Jones, D., 1968. Point of distinction between battery and free range eggs. Nature 220:921.
61. Karapetyan, S.K. & R.A. Arutyunyan, 1966. Change in the morphological properties of eggs depending on environmental temperature. Biol. Zh. Armenii 19:24 (in Russian).
62. Kaufman, L., 1938. Entwicklung und Wachstum von Hühnerembryonen in frischen und gelagerten Eiern. Arch. Geflügelk. 12:65.
63. Kaufman, L., 1939a. An experimental study on the effects of storage on embryonal development of hens' eggs. Proc. 7th World's Poultry Congr.: 186.
64. Kaufman, L., 1939b. 'Aging' of eggs. 1. The influence of storage of hens' eggs on mortality, development and growth of embryos. Pa. państ. Inst. nauk. Gospod. wiejsk. w Pulawach. 17:2.
65. Kaufman, L. & H. Krzanowska, 1957. Investigations of the 'aging' of eggs. III. Influence of egg storage on the weight of the body and of some organs, the hemoglobin content in blood and the histological structure of the thyroid gland of chicks. Roczn. Nauk roln. 72:385 (in Polish).
66. Kazachkina, S.B., 1970. Study of the quality of chicken egg white by the method of analysis of 'white droplet'. Sbornik Rabot molodykh Uchenykh VNITIP 11:138 (in Russian).
67. Keba, V., 1971. The incubation of a batch of eggs of equal age. Ptitvodstvo 8:33 (in Russian).
68. Kleimenova, N.A., 1972. Storage of eggs destined for incubation. Obzor inform. Byull. VNIITEISKh. 4:41 (in Russian).
69. Knize, R. & F. Rydlová, 1965. Genetic aspects of the hatchability of domestic fowl. Živočišná Výroba 10:415 (in Czech).
70. Knorre, A.G., 1959. Brief outline of human embryology with elements of general, comparative and experimental embryology. Leningrad, Medgiz (in Russian).

71. Kolupaeva, T.D., 1970. Influence of vitamin B_{12} on some biochemical parameters of chicken eggs. Sbornik Rabot molodykh Uchenykh VNITIP. 11:300 (in Russian).
72. Kondratenko, I.I., 1970. Morphological and physico-chemical properties of incubation eggs of chickens as related to vary varying levels of energetic-protein nutrition. Sbornik Rabot molodykh Uchenykh VNITIP. 11:145 (in Russian).
73. Korotkova, G.P., 1956. Antibiologic properties of the membrane of white in chicken eggs. Problemy sovremennoi Embriologii 323 (in Russian).
74. Kosin, I.L., 1956. Studies on pre-incubation warming of chicken and turkey eggs. Poultry Sci. 35:1384.
75. Kosin, I.L., 1964. Recent research in hatchability. Related problems of the domestic fowl. World's Poultry Sci. J. 20:254.
76. Kucherova, F.N., 1968. Mineral composition of the membrane of egg white before incubation. Sel'.-khoz. Biol. 3:413 (in Russian).
77. Kucherova, F.N. & S.N. Golubey, 1970. Mineral composition of the chicken egg before incubation. Sel'.-khoz. Biol. 5:617 (in Russian).
78. Kuchkovskaya, E.N., 1938. Hatchability of chicken eggs as related to shell roughness. Sov. Ptitsev. 12:17 (in Russian).
79. Kushner, Kh.F., I.G. Moiseeva & E.V. Tolokonnikova, 1971. The influence of mating chickens of various breeds and lines on egg quality. Geneticheskie osnovy selektsii i gibridizatsii sel'sk.-khoz. ptitsy (tezisy dokladov na simpoziume) 135 (in Russian).
80. Landauer, W., 1967. The hatchability of chicken eggs as influenced by environment and heridity. Storrs agr. Exp. St. Univ. Connecticut.
81. Lea, C.H., 1962. Recent advances in food science. Vol. 1:92. London. Cited by Parkinson (126).
82. Leclercq, B., 1966. Contribution à l'étude du métabolisme des lipides chez l'embryon de poulet; utilisation des réserves vitellines en présence d'acide élaïdique incorporé dans l'oeuf par la poule pondeuse. Annls Biol. anim. Biochim. Biophys. 6: 503.
83. Limarenko, M.N., 1968a. Rational methods for determining the quality of incubation eggs. Sbornik Trudov VNII po Boleznyam Ptits 5(16):104 (in Russian).
84. Limarenko, M.N., 1968b. Physico-biochemical investigations of incubation eggs during storage. Sbornik Trudov VNII po Boleznyam Ptits 5(16):108 (in Russian).
85. Lisovskaya, N.P. & N.B. Livanova, 1960. Fosfoproteiny. Moscow, Izd. AN SSSR.
86. Litko, P.M., 1968. Effect of the weight of incubation eggs on hatchability and post-embryonic development of chicks. Opytnoe Khoz.-vo UNIIP:49 (in Russian).
87. Lomova, A.I., 1939. Determination of the modus of porosity of egg shells in a population of chickens at the association 'Ptitsevod' and at the breeding farm 'Udarnoe'. Trudy Voronezh. zoovet. Inst. 5:92 (in Russian).
88. Lorkiewiczowa, M., 1960-1962. Influence of the duration of

storage of breeder eggs on the growth and viability of chicks and on the productivity of chickens. Annls Univ. Mariae Curie-Sklodowska, E., 15:249 (in Polish).
89. Lutz, H. & Y. Lutz-Ostertag, 1957. Gémellite expérimentale et situs inversus viscerum chez l'embryon d'oiseau. C.r. Acad. Sci. 244:1543.
90. MacLaury, D.W. & W.M. Insko Jr., 1968. Relation of pre-incubation factors and post-hatching performance to length of incubation period. 1. Effects of egg weight and storage time on length of incubation period. Poultry Sci. 47:305.
91. Maesso, E.R., D.V. Vadehra & R.C. Baker, 1969. Chemical composition of egg exterior structures. Poultry Sci. 48:1838.
92. Makarova, Z.N. & S.I. Bogolyubskii, 1969. Nucleic acid content in non-incubated chicken eggs. Sbornik nauchn. Trudov Pushkinsk. nauchno-issled. Lab. Razvedeniya sel'sk.-khoz. Zhivotnykh. 13:254 (in Russian).
93. Marion, W.W., A.W. Nordskog, H.S. Tolman & R.H. Forsythe, 1964. Egg composition as influenced by breeding, eggsize, age and season. Poultry Sci. 43:255.
94. Marion, J.E., J.G. Woodroof & D. Tindell, 1966. Physical and chemical properties of eggs as affected by breeding and age of hens. Poultry Sci. 45:1189.
95. Marshall, W., 1947. The principles of incubation. In: Diseases of poultry. London.
96. Martin, W.G., N.H. Tattrie & W.H. Cook, 1963. Cited by Parkinson (126).
97. Maslieva, O.I., 1950. Vitamin A content in yolk as an indicator of incubation properties of poultry eggs. Trudy nauchno-issled. Inst. Ptitsevodstva 21:121 (in Russian).
98. Marice, D.M. & A. Fidanza, 1954. The structure of the yolk of the hen's egg, investigated by means of its permeability to Br^{82}. J. exp. Biol. 31:573.
99. Merat, P. & L. Lacassagne, 1961. Taux d'éclosion et position de l'oeuf dans la série chez la poule. Annls Biol. anim. Biochim. Biophys. 1:101.
100. Moiseeva, I.G., 1964. Variability and heritability of some features of egg quality in Russkaya Belaya chickens. Trudy Inst. Genet. 31:309 (in Russian).
101. Moiseeva, I.G., 1965. Lipid and cholesterol content in eggs of Russkaya Belaya chickens as related to productivity. Trudy Inst. Genet. 33:119 (in Russian).
102. Moiseeva, I.G., 1970. Influence of inbreeding on the quality of chicken eggs. Genetika 6:99 (in Russian).
103. Moiseeva, I.G. & E.V. Tolokonnikova, 1968. The relation of some quality parameters of chicken eggs and the level of productivity of hens with hatchability. Zhivotnovodstvo 2:54 (in Russian).
104. Moiseeva, I.G. & E.V. Toloknonnikova, 1968. The quality of chicken eggs and hatchability. Zhivotnovodstvo 2 (in Russian).
105. Mongin, P., 1968. Role of acid-base balance in the physiology of egg-shell formation. World's Poultry Sci. J. 24:200.
106. Movchan, N.A., 1967. Some data on the fungicidal properties of the vitelline membrane of eggs. Fitontsidy, ikh biologi-

cheskaya rol' i znachenie dlya meditsiny i narodnogo khozyaistva: 359 (in Russian).
107. Mountney, G.J. & C. Vanderzant, 1957. Relationship of selected egg quality measurements. Poultry Sci. 36:908.
108. Mueller, W.J., 1956. The relationship between water and CO_2 loss during storage and albumen and yolk quality after storage. Poultry Sci. 35:1161.
109. Mueller, W.J., 1959. Factors affecting the quality loss in egg albumen during storage. Poultry Sci. 38:843.
110. Munro, S.S., 1971. Effect of age of hen on albumen height and pH. Poultry Sci. 50:1515.
111. Munro, S.S. & I.L. Kosin, 1945. Proof of the existence of pre-oviposital embryonic dealths in chickens and their bearing on the relation between 'fertility' and hatchability. Can. J. Res. Sect D 23:129.
112. Myand, T., 1969. Influence of breed of chicken, duration of egg storage and weight of the egg on hatchability. Izvestiya Akad. Nauk Estonsk. SSR. 18(3):247 (in Russian).
113. Myannik, E. & I.O. Nymmisto, 1968. The phosphorus content in White Leghorn chicken eggs without shell. Sbornik nauchn. Trudov Estonsk. sel'sk.-khoz. Akad. 58:127 (in Russian).
114. Nady, Z. & H.E. Olah, 1956. Spectroscopic examination of trace elements in the egg shell. Kisérl. Orvostud. 8:516 (in Hungarian).
115. Nagai, Y. & R.S. Gowe, 1969. Genetic control of egg quality. 1. Sources of variation. 2. Selection for maximum rate of improvement. Br. Poultry Sci. 10:337, 351.
116. Needham, J., 1931. Chemical embryology. Cambridge, Univ. Press.
117. Neel, J.K., 1942. A calibration of the development of the chick under improved conditions of incubation. Poultry Sci. 21:294.
118. Nestor, K.E. & K.L. Brown, 1968. Egg traits and hatchability of turkey eggs. Poultry Sci. 47:1700.
119. New, D.A.T., 1956. The formation of sub-blastodermic fluid in hens' eggs. J. Embryol. exp. Morphol. 4:221.
120. Olsen, M.W., 1951. Effect of low temperatures on the hatchability of eggs from various standard bred and crossbred chickens. Poultry Sci. 30:180.
121. Orlov, M.V., 1948. Incubation of eggs stored during various periods. Trudy nauchno-issled. Inst. Ptitsevodstva 19:183 (in Russian).
122. Orlov, M.V., 1954. Selection of fertilized eggs with strong embryos. Trudy VNIIP 24:156 (in Russian).
123. Orlov, M.V. & K.V. Zlochevskaya, 1966. Comparative study of some aspects of embryonic development in chickens of the Cornish Moskovskaya Belaya breed. Trudy VNITIP 30:78 (in Russian).
124. Otrygan'ev, G.K., 1966. Some constitutional pecularities in the embryonic development of chickens. Trudy XIII Vsemirnogo Kongressa po Ptitsevodstvu, Kiev: 530 (in Russian).
125. Otrygan'ev, G.K., 1968. Problems of contemporary incubation. Ptitsevodstvo 1:22 (in Russian).

126. Parkinson, T.L., 1966. The chemical composition of eggs. J. Sci. Food Agric. 17:102.
127. Payne, L. & R. McDaniel, 1958. Shell thickness as related to 'shuck-outs' in turkey eggs. Poultry Sci. 37:825.
128. Pel'ttser, S.O., 1966. Enhancement of breed and productivity qualities of chickens by improving conditions of embryonic development. Materialy i Rekommendatsii Vsesoy. Konf. Uluchsh. plemennogo Dela v Zhivotnovodstve: 499 (in Russian).
129. Pel'ttser, S.O., 1969. Year-round incubation of chicken eggs. Author's summ. of Doct. Diss. Moscow (in Russian).
130. Perutz, M.F., 1967. A discussion on the structure and function of lysozyme. Proc. R. Soc. Ser.B. Biol. Sci. 167: 348.
131. Pigarev, N.V., L.A. Makagon & N.V. Nikolotova, 1966. Reproductive capacities of chickens in the first year of lay and year-round production of incubation eggs. Trudy VNIIptitsepere-rabatyvayushchei Promyshlennosti 12:120 (in Russian).
132. Pingel, H., 1958. Untersuchungen über Beziehungen des Gewichts und der Schalenfarbe von Bruteiern zur Schlupffähigkeit bei der Rasse New Hampshire. Dt. GeflZtg 7:23/24, 354.
133. Pingel, H. & M. Bock, 1969. Die Abhängigkeit von Eimasse, Schalenstärke und Eiklarindex von der Stellung des Eies in der Legeserie. Arch. Geflügelz. Kleintierk. 18:61.
134. Pletsityi, D.F. & V.G. Krasyanskaya, 1963. Change in lysozyme activity in the white of chicken eggs during embryogenesis. Dokl. Akad. Nauk SSSR 149:478 (in Russian).
135. Potokina, L. & A. Protsenko, 1970. Aero-ionization of incubation eggs. Ptitsevodstvo 5:35 (in Russian).
136. Rauch, W., 1952. The influence of eggshell porosity on the number of chickens hatched from incubating eggs. Poultry Sci. 31:589.
137. Reinke, W.C. & R.C. Baker, 1966. Relationship between carbon dioxide permeability and bacterial penetration in chicken egg shell models. Poultry Sci. 45:1327.
138. Riley, D.P., 1953. The chemistry of the egg. Proc. R. Instn Gt Br. 35(11):363.
139. Rogulska, T. & A. Komar, 1969. The relationship between the orientation of the early chick embryo and the shape of the egg shell. Experientia 25:990.
140. Rol'nik, V.V., 1955. Incubation of eggs of the eider (Somateria mollissima L). Zool. Zh. 34:885 (in Russian).
141. Rol'nik, V.V., 1968. The biology of embryonic development in birds. Nauka, Lemingrad (in Russian).
142. Romanoff, A.L. & A.J. Romanoff, 1949. The avian egg.
143. Sauter, E.A., C.F. Peterson & E.E. Steele, 1970. The relationship of the lysozyme fraction in thick egg white to fertility and hatchability of eggs. Poultry Sci. 49:987.
144. Sauveur, B., 1967. Conservation des oeufs de poule et éclosivité. Essai de comparaison avec les données obtenues sur la conservation des oeufs de consommation. Annls Zootech. 16:89.
145. Sauveur, B., R. Ferre & L. Lacassagne, 1967. Conservation d'oeufs de poule sous atmosphère enrichie en gaz carbonique. Annls Zootech. 16:351.

146. Schneider, K.H., 1969. Untersuchungen über die Beziehungen einiger Eiqualitätsmerkmale zum Brutergebnis. Arch. Geflügelz. Kleintierk. 18:69.
147. Shalimovich, V.N., 1955. Study of the structure of the yolk membrane in chicken eggs with fluorescent and ultraviolet microscopy and some histochemical methods. Dokl. Akad. Nauk SSSR 105:584. (in Russian).
148. Shevtsova, M. Ya., 1965. Growth and respiration in the embryonic period of embryogenesis in the domestic duck. Materialy Simpoziuma osnovym Problemam vozrastnoi Fiziologii Biokhimii. Kharkov. 201 pp. (in Russian).
149. Sheinis, S.A., 1964. Nutrition of the avian embryo at various stages of development. Problemy sovremennoi Embriologii. Univ. Press, Leningrad. 361 pp. (in Russian).
150. Shishkina, E.M., 1949. Changes in embryogenesis of birds under the influence of egg aging and incubation temperature. Dolk. mosk. el'.-khoz. Akad. K.A. Timiryazeva 9:130 (in Russian).
151. Shkeir, S.D., 1970. Hydrogen ion concentration in incubating eggs stored with gamma irradiation. Radiobiologiya 10(3):437 (in Russian).
152. Shkredov, V.M., 1971. Pre-incubation enrichment of egg plasmas with carbonic acid as a means of raising the hatching rate. Author's summary, Cand. Diss., Omsk (in Russian).
153. Shpits, I.S., 1966. The role of the shell of the chicken egg in the metabolism of minerals in the developing embryo. Trudy mosk. vet. Akad. 49:139 (in Russian).
154. Shpits, I.S. & A.K. Danilova, 1966. The effect of shell quality of the incubation egg on minerals metabolism in the embryo and the adult bird. Trudy VNITIP 30:87 (in Russian).
155. Shpits, I.S., V.I. Kobzeva & A.K. Danolova, 1965. Shell thickness and incubation properties of eggs. Ptitsevodstvo 11:20 (in Russian).
156. Shtele, A.L., 1970. Incubation properties of White Leghorn chicken eggs after Hybridisation. Dokl. VASKhNIL 9:33 (in Russian).
157. Silin, E.K. & L.D. Kikavskii, 1969. The effect of inbreeding on hatchability, live weight and viability of Cornish and Plymouth Rock pullets. Trudy VNITIP 34:10 (in Russian).
158. Simons, P.C.M. & G. Wiertz, 1966. Ultrastructure of the cuticular surface of the chicken as related to cleaning. Abstract. Trudy XIII Vsemirnogo Kongr. Ptitsevodstvu. Kiev. 631 pp. (in Russian).
159. Sittmann, K., H. Ablanalp & C.F. Meyerdick, 1971a. Extended storage of quail, chicken and turkey eggs. 1. Hatchability and embryonic mortality. Poultry Sci. 50:681.
160. Sittmann, K., H. Ablanalp & U.K. Abbott, 1971b. Extended storage of quail, chicken and turkey eggs. 2. Embryonic abnormalities and the inheritance of twinning in quail. Poultry Sci. 50:714.
161. Smith, A.Y.M., 1931. Movement of water in the hen's egg. Dept. Sci. Ind. Res., Food Invest. Rep. 161 pp. Cited by Mueller (109).

162. Smith, M.B. Cited by Parkinson (126).
163. Snyder. Facteurs actuellement connus pour influencer la qualité de l'oeuf. Cited by Sauveur (144).
164. Steinke, L., 1966. Versuche mit Methoden zur Verbesserung der Schlupffähigkeit von Bruteiern. Arch. Geflügelk. 3:192.
165. Sturkie, P.D. & A.G. Williams, 1945. Studies on pregastrula development, early embryonic development and hatchability of pre-maturely laid eggs of the hen. Poultry Sci. 24:545.
166. Svensson, S.A., 1957. Der Einflusz der Beschaffenheit der Eierschale auf die Brutfähigkeit von Hühnereiern. Arch. Geflügelk. 21:145.
167. Swartwood, J. & J.V. Spencer, 1967. The effects of carbon dioxide and oxygen on the physical properties of shell eggs. Poultry Sci. 46:1326.
168. Tarabrina, L.G., R.S. Gafurov, T.G. Mitina & M.M. Pirogova, 1968. Study of the morphological structure of chicken eggs as related to age and season. Trudy ul'yanov. sel'.-khoz. Inst. 13:219 (in Russian).
169. Tolokonnikova, E.V., 1964. Variability of morphological parameters in chicken eggs and their relation with nutritive and incubation qualities of eggs. Trudy Inst. Genet. 31:309 (in Russian).
170. Tolokonnikova, E.V., I.G. Moiseeva & L. Lazberg, 1970. Correlation of egg productivity features in chickens. Ptitsevodstvo 2:19 (in Russian).
171. Toshkov, A. & L. Shirova, 1970. Farmaco Ed. sci. 25:842.
172. Tsarenko, P.P., 1969a. Storage periods for incubating eggs. Zap. leningr. sel'.-khoz. Inst. 112:197 (in Russian).
173. Tsarenko, P.P., 1969b. Selection of eggs by optical density. Zap. leningr. sel'.-khoz. Inst. 112:189 (in Russian.
174. Tsarenko, R.G. & P.P. Tsarenko, 1970. The enhancement of incubation qualities of eggs of broiler lines in the conditions of a warm climate. Materialy II Konf. molodykh uchenykh Genetike Razvedeniyu sell'.-khoz. Zhivotnykh VNII Razvedeniya Genetiki sel'.-khoz. Zhivotnykh. 1:185 (in Russian).
175. Turevskii, A.A., S.F. Baranetskii & T.S. Dolmatov, 1967. Intravitam staining of chicken embryos. Ptitsevodstvo; resp. mezhved. tem. nauchn. Sbornik 4:23 (in Russian).
176. Vadehra, D.V. & R.C. Baker, 1968. Role of the cuticle in egg spoilage. Poultry Sci. 47:1728.
177. Vadehra, D.V., K.R. Nath & R.C. Baker, 1971. Physicochemical properties of the proteins of egg shell membranes. Poultry Sci. 50:1638.
178. Variagiris, A., 1967. Effect of egg white on embryonic development. Trudy litovsk. vet. Akad. 8:325 (in Russian).
179. Veitsman, L., B. Zaitsev, B. Skukovskii, E. Karachenkova & S. Pavlova, 1970. Morphologic and biochemical peculiarities of fowl eggs. Ptitsevodstvo (8) 16 (in Russian).
180. Vladimirova, Yu.N., 1954. Weight losses of chicken eggs before incubation. Trudy nauchno-issled. Inst. Ptitsev. 24: 126 (in Russian).
181. Vladimirova, Yu.N., 1962. Enhancement of embryo viability of

geese during prolonged egg storage. Sbornik Ref. Rabot VNIIP 67 (in Russian).
182. Vladimirova, Yu.N. & A.M. Sergeeva, 1967. Assessment of incubation properties of eggs from layer and broiler chickens. Trudy VNIIP 32:138 (in Russian).
183. Vladimirova, Yu.N. & A.M. Sergeeva, 1968. The standardization of incubation properties of eggs. Mater. vses. nauchno Soveshch. Konf. 1:71 (in Russian).
184. Vladimirova, Yu.N. & A.M. Sergeeva, 1969a. Assessment of incubation properties of eggs. Kolos, Moscow (in Russian).
185. Vladimirova, Yu.N. & A.M. Sergeeva, 1969b. Assessment of incubation eggs by carotene content and yolk colour. Trudy VNITIP 33:54 (in Russian).
186. Vladimirova, Yu.N. & A.M. Sergeeva, 1969c. Staining intensity of the egg shell of Plymouth Rock broilers and its relation to hatchability. Sbornik Ref. Rabot VNITIP (koordiniruemye temy): 98 pp. (in Russian).
187. Vladimirova, Yu.N. & A.M. Sergeeva, 1971. Bird eggs. Spravochnik po inkubatsii yaits sel'skohozyaistvennoi ptitsy. Moscow (in Russian).
188. Vorob'ev, V.I., 1960. Phosphorus compounds in the chicken egg and their conversions during incubation. Voprosy tsitologii. Akad. Nauk SSSR, Moscow-Leningrad. 156 pp. (in Russian).
189. Walden, C.C., I.V.F. Allen & P.C. Trussell, 1956. The role of the egg shell membranes in restraining the entry of microorganisms. Poultry Sci. 35:1190.
190. Weisbroth, S.H. & I.L. Kosin, 1966. Gross spatial changes in the turkey blastoderm following extended pre-incubation storage. Proc. Soc. exp. Biol. Med. 121:795.
191. Wilcox, Jr., F.H. & R.K. Cole, 1954. Studies on the lysozyme concentration in the egg white of the domestic fowl. Poultry Sci. 33:392.
192. Wolken, J.J., 1951. Structure of hen's membranes. Anat. Rec. 111:79.
193. Wolken, J.J. & Schwartz, 1948. Physical structure of shell membranes. J. gen. Physiol. 32:153.
194. Zawalsky, M., 1962. The effect of sex, egg weight and pre-incubation storage on hatching time and chick weight. Poultry Sci. 41:1697.
195. Zlochevskaya, K.V., 1962. Storage in various seasons of eggs obtained from laying hens of varying ages. Sbornik Ref. Rabot Vsesoyuzn. nauchno-issled. Inst. Ptitsevodstva 89:89
196. Zlochevskaya, K.V., 1965. Intensity of development of chicken embryos on the first day of incubation and their subsequent readiness for hatching. Trudy VNITIP 31:126 (in Russian).
197. Zusman, I.N., 1968. Variability of chicken embryos at oviposition and its relation to the rate of subsequent development. Zool. Zh. 47:407 (in Russian).
198. Zusman, I.N., 1969. Variability of chicken blastodiscs in the period between oviposition and the start of incubation and its effect on the rate of subsequent embryonic development. Zool. Zh. 48:1965 (in Russian).

Additional bibliography

1. Agguri el Saliya, 1973. Effect of times and methods of storage in spring on some physico-chemical parameters of chicken eggs of lines 18 and 63. Sbornik nauchn. Trudov mosk. vet. Akad. 71:168 (in Russian).
2. Belcheva, S.Ya., 1974. The cytoplasmic layer in the chicken egg and its change during 'aging'. Aktual'nye Probl. Razvitiya Ptitsevodstva. 7:55 (in Russian).
3. Borisov, S.I., 1970. Changes in protein composition in chicken eggs with various storage methods. Sbornik Rabot molodykh Uchenykh VNITIPtitsevodstva 12:79 (in Russian).
4. Dańczak, A., 1973. Influence of age of the flock of laying hens on the quality of chicken eggs. Drobiarstwo 21(10):6 (in Polish).
5. Danilova, A.K. & I.S. Shpits, 1975. Egg-shell formation and the enhancement of its strength. Vest. sel'.kokh. Nauki 9:51 (in Russian).
6. Eremeev, G.P., M.P. Berezovskaya, G.P. Bol'shakova, V.P. Eliseeva, R.A. Zorik, T.M. Kochkina, T.M. Okolelova & V.I. Parfenova, 1975. The significance of xantophylls for embryonic development of birds. Nauch. Trudy Omsk. sel'.kokh. Inst. 128:59 (in Russian).
7. Halai, M. & S. Kopernicky, 1975. Study of the quality of chicken eggs as related to storage time and temperature. Part 1. Effect of storage time and temperature on weight loss and changes in the percentual proportion of egg components. Polnohospodarstvo 21:248 (in Slovak with Russ. and Eng. summary).
8. Kleimenova, N.A., 1973. The effect of modified storage conditions on morphologic and physico-chemical parameters of eggs. Materialy vsesoyuzn. nauch. Konf. VNITIPtitsevodstva 6:195 (in Russian).
9. Klieste, E., 1971. Effect of crystalline vitamins on incubation parameters of Plymouth Rock chicken eggs. Khimiya Biol. sel'sk.-khoz. 1:64 (in Russian).
10. Klieste, E.M., 1972. Effect of supplementation with group B vitamins on the quality of Plymouth Rock breeder eggs. Nauka Zhivotnovodstvu 11:59 (in Russian).
11. Kromin, V. & N. Fatikova, 1974. Effect of a gaseous nitrogen environment on incubation properties of eggs. Ptitsevodstvo 3:28 (in Russian).
12. Lepaiye, L., E. Vyagi & E. Myannik, 1975. Age dynamics of the morphology and chemical composition of Leghorn eggs in conditions of industrial poultry farming. Sbornik nauchn. Trudov est. sel'sk.-khoz. Akad. 96:41 (in Estonian with Russian and English summary).

13. Mašić, B., N. Apostolov, L. Srajber & L. Žigić, 1974. Effect of egg weight on growth and quantitative genetic parameters of White Plymouth Rock chicks. Arh. poljopr. Nauke 27:99, 159 (in Serbocroat with English summary).
14. Neifel'd, E.G. & L.K. Lepaiye, 1974. Lecithin and cholesterol contents of chicken eggs. Sb. nauch. Trudov est. sel'sk. khoz. Akad. 80:129 (in Russian).
15. Potokina, L.I., 1971. Growth and development of chicks obtained from eggs which had been stored at constant aeroionization. Trudy mosk. vet. Akad. 57:132 (in Russian).
16. Sabo, V. & J. Staško, 1972. Effect of preliminary warming and storage of chicken eggs on hatchability and embryo viability. Ved. Pr. výzk. Ust. Chov hyd. Ivanke pri Dunaji 12:105 (in Slovak).
17. Sharlanov, D., K. Kunev & N. Bachev, 1971. Effect of size of turkey eggs on hatchability and on weight development of hatched turkey poults. Zhivotnov. Nauki 8(1):77 (in Bulgarian).
18. Shatokhina, S.T., 1973. Relationship between some morphological and physico-chemical properties of chicken eggs, and hatchability. Trudy kuban. sel'sk.-khoz. Inst. 76(104):21 (in Russian).
19. Shpits, I.S., 1971. Dependence of egg-shell quality on metabolic activity of the chicken skeleton. Trudy mosk. vet. Akad. 57:181 (in Russian).
20. Tsarenko, P.P., 1971. Shape index as a parameter of marketing and incubation qualities of (chicken and duck) eggs. Zap. leningr. sel'.-khoz. Inst. 141:207 (in Russian).
21. Tsarenko, P.P., 1974. Shape index as a selection marker. Ptitsevodstvo 8:25 (in Russian).
22. Tsarenko, P.P., A.I. Samygina, A.G. Spiridonov & Kh.A. Randalainen, 1974. Effect of environmental conditions on the quality of chicken eggs. Zap. leningr. sel'.-khoz. Inst. 204: 162 (in Russian).
23. Vladimirova, Yu.N., 1971. Hatching rate as related to egg position during storage. Ptitsevodstvo 4:29 (in Russian).
24. Zelnic, J., J. Bulla & J. Granat, 1970. Morphology of germinal discs in fertilized eggs of the Japanese quail (Coturnix c. japonica). Part II. Polnohospodarstvo 16:958 (in Slovak).
25. Burkhardt, A.D., 1974. Qualitative Eigenschaften von Eiklar und Eigelb und ihre Beeinflussung durch Tieralter und Lagerungsdauer. Dissertation Stuttgart-Hohenheim.
26. Chahil, P.S. & W.A. Johnson, 1974. Effect of preincubation storage, parental age, and rate of lay on hatchability in Coturnix coturnix japonica. Poultry Sci. 53:529.
27. Cherms, F.L. & J.J. MacIlraith, 1974. Long term storage of turkey hatching eggs. In: XV World's Poultry Congress and Exposition. Proceedings and abstracts: 329.
28. Cristensen, V.I. & N.P. Johnston, 1975. The effect of time of day of insemination and oviposition on the fertility of turkey hens. Poultry Sci. 54:1209.
29. Cristie, W.W. & J.H. Moore, 1972. The lipid composition and triglyceride structure of eggs from several avian species. Comp. Biochem. Physiol. B 41:297.

30. Dhaliwal, J.S. & R.M. Acharya, 1972. Relationship of egg weight and egg quality with hatchability. Punjab Agric. Univ. J. Res. 9, Supp. 1:224.
31. Gardner, F.A. & L.L. Young, 1972. The influence of dietary protein and energy levels on the protein and lipid content of the hen's egg. Poultry Sci. 51:994.
32. Gentry, R.F. & C.L. Quarles, 1972. The measurement of bacterial contamination on egg shells. Poultry Sci. 51:930.
33. Härtel, H., 1972. Zur Bedeutung der essentiellen Fettsäuren, in besondere der Linolsäure, für die Ernährung von Hühnern. Kraftfutter 55:623.
34. Hussaini, S.S.H. & A.H. Rizvi, 1972. Effect of date of hatch on age at first egg in White Leghorn pullets. Indian vet. J. 49:678.
35. Ismail, P.K. & S.G. Nair, 1972. A note on the amino acid composition of albumen and yolk from duck and hen eggs. Indian J. Poultry Sci. 7:43.
36. Kiefer, H., 1973. Der Einfluss des Hennenalters auf Eigewicht und Zusammensetzung des Eies. Hohenheim Arb. Schriftenr. Univ. Hohenheim 72:26.
37. Koeler, H.H., 1974. Physicochemical appraisal of changes in egg white during storage. J. agric. Food Chem. 22:288.
38. Kosin, I.L. & T. Konishi, 1973. Preincubation storage conditions and their effect on the subsequent livability of chicken embryos: exogenous CO_2, plastic bags and extended holding periods as factors. Poultry Sci. 52:296.
39. Lasar, V., L. Kriz, F. Špaček & P. Straka, 1974. Change in various characters of turkey incubation eggs during storage. Živočišna Výroba 19(8):601 (in Czechoslovak).
40. Merrit, 1970. Hatching egg storage and postembryonic performance. Poultry Sci. 49(1):316.
41. Momma, H., M. Nakano & Y. Fujino, 1972. Studies on the lipids of egg yolk. Jap. J. zootech. Sci. 43(4):198.
42. Munro, S.S., 1971. Effect of age of hen on albumen height and pH. Poultry Sci. 50(5):1515.
43. Narasimha, R.B., R.M. Michael & P. Kothandaraman, 1972. Study of some factors related to hatchability in white Leghorn eggs. Effect of preincubation storage conditions on hatchability. Indian Vet. J. 49(8):800.
44. Nasin, A.K., A. Chaudhry & M.B. Sial, 1972. Comparative study on hatchability, fertility and embryonic mortality in the first and second years of White Leghorn and Layllpur Silver Black breeds. J. agric. Res. 10(1):54.
45. Nasim, A., A. Manzur-ud-Din & M.R. Chaudhry, 1973. Influence of age and breed of chicken on the chemical composition of egg yolk. Pakist. J. Sci. 25(4-6):183.
46. Oluyemi, J.A. & O. George, 1972. Some factors affecting hatchability of chicken eggs. Poultry Sci. 51(5):1762.
47. Parkinson, J.T.L., 1972. Separation of the proteins of egg white and egg yolk and a study of their interactions in whole egg. 1. Quantitative fractionation of proteins. 2. Lipid and phosphorus contents and molecular weights of some fractions. 3. Amino acid composition of protein fractions. 4. Electro-

phoretic studies. J. Sci. Food Agric. 23(5):649.
48. Proudfoot, F.G., 1972. Influence of an improved hatching - egg storage method on the subsequent performance of broiler chickens. Can. J. Anim. Sci. 52(2):203.
49. Proudfoot, F.G., 1974. Effects of genotype and storage time on hatchability when chicken eggs are in contact or separated during hatching. Can. J. Anim. Sci. 54(4):713.
50. Reinke, W.C., J.V. Spencer & L.J. Trynhew, 1973. The effect of storage upon the chemical, physical and functional properties of chicken eggs. Poultry Sci. 52(2):692.
51. Sato, Y., K. Watanabe & T. Takahashi, 1973. Lipids in egg white. Poultry Sci. 52(4):1564.
52. Sittmann, K. & H. Ablanalp, 1971. Extended storage of quail, chicken and turkey eggs. 3. Tertiary sex ratio in quail. Poultry Sci. 50(3):722.
53. Smith, K.P. & B.B. Bohren, 1975. Age of pullet effects on hatching time, egg weight and hatchability. Poultry Sci. 54 (4):959.
54. Steinke, L., 1975. Untersuchungen an kurzfristig bebrüteten Hühnereiern. Arch. Geflügelk. 39(4):142.
55. Winn, S.E. & H.R. Rall, 1973. β-N-acetylglucosaminidase activity in the membranes of the chicken egg. Poultry Sci. 54(5):2104 abstr.
56. Woodford, R.C., 1972. Producing and handling high quality eggs. S. Aust. Dep. Agric. Ext. Bull. 28.