In vitro experiments using sacs of rat, guinea pig and rabbit intestine filled with glucose-1-^{14}C and incubated in Krebs-Ringer phosphate at 37° C showed that under the condition of these experiments only a small fraction, usually less than 1/5, of the glucose disappearing from the lumen could be detected in the surrounding fluid. Practically all the radioactivity appearing in the outside fluid after periods of incubation from 30–60 min could be accounted for as glucose. Randomisation of ^{14}C determined by degradation as previously described, was small and always less than 20%.

It is suggested that these experiments and most of those of other workers may be explained on the assumption that the major portion of glucose is normally absorbed *in vivo* without degradation and that the remainder is metabolised to provide energy for the absorption. The conditions of *in vitro* studies of intestine may encourage considerable degradation without concomitant transport.

These findings are in broad agreement with recent reports[10,11], which were published while these experiments were in progress.

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Nucleic acid content of the egg of the domestic fowl

Early work on nucleic acids in unincubated eggs has been discussed by BRACHET[1] and recent literature on deoxyribonucleic acid (DNA) in eggs has been reviewed by HOTCHKISS[2]. Recently DNA has been measured in the whole hen's egg[3] and FRAENKEL-CONRAT *et al.*[4] isolated avidin, a protein of hen's egg white and found that it contained DNA. Further quantitative evidence for the presence of both ribonucleic acid (RNA) and DNA in egg white and yolk using an isotope dilution technique is reported here.

Unincubated egg yolk and white (from Rhode Island Red hens) from 6–10 eggs were separately homogenized in water with a Waring blendor. Free nucleotides were removed from these homogenates by precipitation of protein with perchloric acid (0.2 *M* at 0° C), and after removal of lipid with ethanol and ether, nucleic acids were extracted with 1.0 *M* perchloric acid at 70° C for 1 hour. The method of KIRBY[5,6] for obtaining nucleic acid extracts was also used: sodium *p*-aminosalicylate (6% w/v) was dissolved in a homogenate of egg white or yolk and the mixture then stirred with an equal volume of 90% (w/v) aqueous phenol solution for 1 hour. After centrifugation, the upper layer was removed and dialysed against distilled water for 2 days at 0° C (after centrifugation of yolk homogenates the upper aqueous and lipid layers were removed and the lipid extracted with ether before dialysis).

RNA in these extracts was measured by an isotope dilution method for uracil[7], and DNA by the same method for thymine[8]. RNA was also determined by the orcinol method[9] after removal of free hexoses[10], and DNA by reaction of indole with the deoxyribose component[11], with a correction for absorption at 520 mμ[12]; neither of these colorimetric methods produced reliable results in extracts of egg yolk or white. Previously reported[12] amounts of DNA in egg yolk and white based on the latter colour reaction are probably erroneous owing to colour contamination.

Aliquots of perchloric acid extracts were concentrated under reduced pressure to 70% (w/v) perchloric acid. Aliquots of nucleic acid extracts obtained by the phenol method were evaporated to dryness and suspended in 70% (w/v) perchloric acid. Pyrimidines were liberated from the nucleic acids by digestion with 70% (w/v) perchloric acid for 1 hour at 100° C, when either

thymine-2-^{14}C or uracil-2-^{14}C was added and the perchloric acid precipitated with KOH. The pyrimidine was then purified by running the whole digest on at least four successive paper chromatograms[13]. By comparison of the initial and final specific activities (counts per min/μg) of the pyrimidine, the amount of pyrimidine present in the extract was determined. Results are based on nucleic acid preparations obtained from rat liver by the method of KIRBY[5,6]; the uracil content of the RNA was 6.3% and the thymine content of the DNA was 10.9%. Counting and ultraviolet absorption errors were experimentally determined and are expressed as coefficients of variation in Tables I and II.

TABLE I

"ACID-SOLUBLE" URACIL AND THYMINE DERIVATIVES IN EGG WHITE AND YOLK

	Uracil (μg per white or yolk)	*Thymine (μg per white or yolk)*
White	460 ± 55	19 ± 4
Yolk	13 ± 1	6.6 ± 0.3

TABLE II

NUCLEIC ACIDS IN EGG WHITE AND YOLK

	RNA (μg per white or yolk)	*DNA (μg per white or yolk)*
Yolk		
Perchloric acid extraction	68 ± 14	52 ± 6 57 ± 7
Phenol partition	114 ± 14	39 ± 2
White		
Perchloric acid extraction	520 ± 33	308 ± 16
Phenol partition	2260 ± 100 1595 ± 50	137 ± 30 142 ± 5

The amounts of "acid-soluble" uracil and thymine derivatives are given in Table I; as the acid-soluble extract was digested with 70% (w/v) perchloric acid, these figures represent total free pyrimidine and pyrimidine nucleotide material. The nucleic acid content of egg white and yolk is given in Table II. The RNA content of the white is very much greater than that of the yolk, and the phenol partition method appears to extract more RNA than the perchloric acid method. Perchloric acid appears to give a better extraction of DNA than the phenol method; however, this may be due to insufficient washing of the phenol layer. The ratio of amounts of nucleic acid in egg white and yolk (obtained by the phenol method) to amounts of the respective pyrimidine in the "acid-soluble" fraction is approximately constant (6.5).

Using a microbiological method for deoxyriboside (after depolymerisation of DNA with deoxyribonuclease), HOFF-JØRGENSEN[3] estimated that the unincubated whole hen's egg contained 118 ± 12 μg DNA. This is somewhat lower than figures obtained by the isotope dilution method with perchloric acid extraction (362 μg) and the phenol method (179 μg DNA). FRAENKEL-CONRAT *et al.*[4] using the method of SCHNEIDER[14] found 30–45 μg total nucleic acids per ml of egg white; this is roughly equivalent to 900–1350 μg nucleic acid per egg white. Average figures for the total nucleic acid in egg white in this work are close to this range (828 μg by the perchloric acid method and 2068 μg by the phenol method).

As egg white is a secretion of the oviduct, it is not surprising to find large quantities of RNA present and the DNA may be derived from cell debris in the oviduct. The amount of "cytoplasmic DNA" in the yolk represents a great excess over nuclear DNA in the early blastoderm as even after 24 h incubation a blastoderm contains only about 3 μg DNA[12].

It is possible that this "cytoplasmic DNA" and RNA (or similar highly polymeric compounds) are a convenient means of forming a storage reserve of nucleic acid precursors which could be obtained by degradation when required by the embryo.

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Complex formation between bovine serum albumin and sodium deoxyribonucleate induced by heat

GREENSTEIN AND HOYER[1] observed that under special conditions, deoxyribonucleic acid (DNA) prevents the heat coagulation of serum albumin. The special conditions are: (1) The mixture of albumin and DNA must be practically salt free. (2) The pH of the mixture must be adjusted to about 5.3 prior to heating. Nearly complete protection of the albumin solutions against heat coagulation is afforded by 0.5 to 1% by weight of DNA relative to albumin. The question of the mechanism of protection was left open although in a note[2] immediately following the GREENSTEIN paper it was suggested that binding was probably not involved since two peaks were observed in the ultracentrifuge after heating.

Subsequently GEIDUSCHEK AND DOTY[3] investigated the interaction of bovine serum albumin (BSA) and DNA by light scattering at room temperature. Their procedure was to mix the DNA and BSA together and to measure the light scattering by diluting this mixture into a phosphate buffer which served as solvent. Various pH's were studied. At pH 7.47 and 6.46, no reaction was observed. At pH 5.51, DNA was found to bind 35% of its own weight of BSA. The authors felt that despite the differences between the conditions of the two experiments, the amount of binding displayed was quite inadequate to explain the protective action of DNA on BSA. It was suggested that the stronger binding that such protection appeared to require may only occur with parts of the protein structure that may be made accessible by denaturation.

This investigation represents our search for this stronger type of binding that is thought to occur.

The bovine serum albumin was a crystalline product obtained from Pentex Incorporated, Kankakee, Illinois. The deoxyribonucleic acid was prepared from salmon sperm by Dr. NORMAN SIMMONS. All solutions were heated in small glass stoppered test tubes by immersing in boiling water for ten minutes. Solutions prepared for heating experiments contained about 0.5 g BSA/dl. The albumin stock solution was prepared by the method of GREENSTEIN AND HOYER[1]. The DNA stock solution was prepared by diluting a concentrated solution in distilled water.

It was at once apparent that the products of heating are a sensitive function of the pH. If albumin alone is heated at pH 5.5, partial heat coagulation occurs as evidenced by formation of a milky opacity in the solution. However, analytical ultracentrifugation (Spinco Model E) shows that there is considerable albumin remaining in solution with the same sedimentation constant as native material. As a result of the heating, the pH rises from 5.5 to about 6.9. If one heats native albumin at pH 6.5, no heat coagulation occurs. But upon heating the albumin at pH 5.4 in 0.0015 M citrate buffer, the pH does not rise above 5.9 and all of the albumin is coagulated. Thus it appears that one of the prerequisites for heat coagulation is that the electrostatic repulsion of the albumin molecules be minimized. As the pH becomes higher, the negative charge on the albumin rises* (note: the isoelectric point of BSA is 4.5) and apparently the repulsion

* The increase in negative charge between pH 5.5 and pH 6.5 is probably accounted for by the neutralization of the imidazole groups of histidine which would be expected to titrate in this region. There are 18 histidine residues per molecule of BSA according to G. R. TRISTRAM, in H. NEURATH AND K. BAILEY, *The Proteins*, Vol. IA, Academic Press, Inc., New York, 1953, p. 215.