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CHANGES IN SPECIFIC SEQUESTRATION OF PROTEIN DURING TRANSPORT INTO THE DEVELOPING OOCYTE OF THE CHICKEN†

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SUMMARY

Coinjection of pairs of ^{125}I- and ^{131}I-labelled proteins into the circulation of laying hens, demonstrated that not all stages in oocyte development had the same capacity to accumulate the injected proteins. With increasing size, up to 200 mg wet weight, small oocytes accumulated IgG (or γ-livetin) at rates which did not parallel those for other proteins: after 200 mg a parallelism was apparent.

INTRODUCTION

In our continuing investigation of the sequestration of proteins by cells we are using the developing oocyte of the hen chicken as an experimental system. Previous investigators have shown that proteins bearing a marker, and injected into the blood of a laying hen, will accumulate in the oocytes[1], or eggs subsequently laid[1,2]. It is also known that proteins found in the oocyte cytoplasm and derived from the blood, may be in different proportions in the two compartments[3]. The earlier work of Patterson *et al.*[1], indicated that there is some differential change in the accumulation of certain serum proteins during the last few days prior to ovulation. The experiments of this report indicate that there is a very marked change in the accumulation by the oocyte during early stages in its development, of IgG derived from the circulation.

MATERIALS AND METHODS

Phosvitin was prepared by the methods of Mecham and Olcott[4] and chromatographed on DEAE-cellulose[5]. Chicken IgG was prepared by repeated precipitation of serum with 33% saturated $(NH_4)_2SO_4$ and subsequent molecular sizing on a Biogel P-300 column. Hemocyanin (spec. act. 10 μCi/mg) was a gift of Professor S. J. Singer and was prepared by the method of Weigle[7]. γ-Livetin (spec. act. 50 μCi/mg) was obtained from a livetin fraction[8], treated as described for IgG.

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Conalbumin (spec. act. 50 μCi/mg) was prepared as described by Kabat and Mayer[9], and was subsequently fully charged with iron. All proteins were iodinated by the Chloramine T method[6], and freed from unbound iodine by passage over a Biogel P-10 column.

To determine the concentration of phosvitin and γ-globulin in the serum and yolk, the proteins were isolated and purified from yolk or serum by standard methods indicated above. Yields are based on recoveries of radioactivity after the initial addition of an ^{125}I trace amount of that protein to either the yolk fraction or the serum.

RESULTS AND DISCUSSION

The accumulated radioactivity in the oocytes, 4 h after intravenous injection of trace amounts of ^{131}I-labelled IgG and ^{125}I-labelled phosvitin into the circulation of a laying white leghorn hen is recorded in Fig. 1. Both labelled proteins are sequestered by the oocytes. The proteins were isolated from the egg and the radioactivity shown to co-chromatogram only with the particular proteins injected. No iodine was detected on other proteins or peptides nor as free I_2. Thus, no detectable degradation of the labelled proteins occurs during the time course of our experiments once the proteins are deposited in the ooplasm.

Having established that the radioactivity remained on the assimilated proteins, we were able to look at the ratios of ^{125}I- to ^{131}I-tagged proteins in a large number of

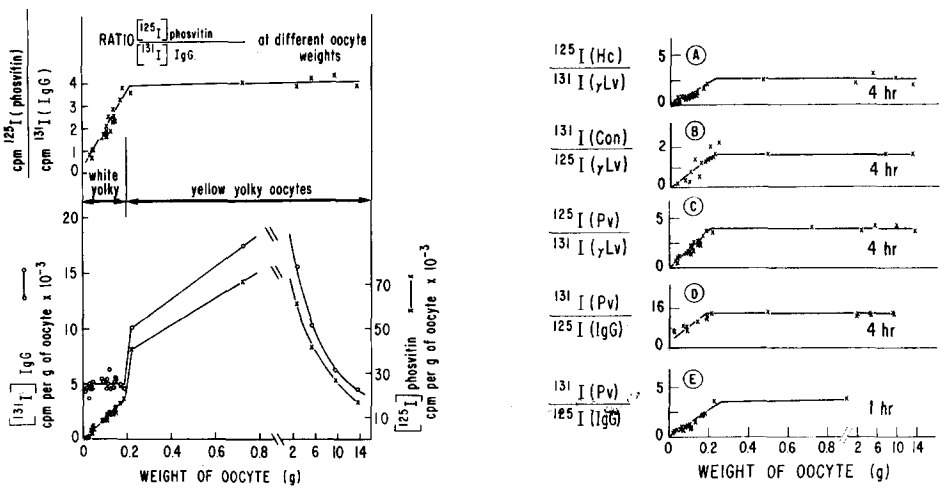

Fig. 1. Accumulation of injected ^{131}I-labelled IgC and ^{125}I-labelled phosvitin by laying hen oocytes. 50 μCi each of ^{131}I-labelled IgG (spec. act. 50 μCi/mg) and ^{125}I-labelled phosvitin (spec. act. 50 μCi/mg) were introduced intravenously into a laying hen. After 4 h, the hen was exsanguinated and the ovary excised. All oocytes greater than 30 mg were removed, repeatedly washed in saline, blotted dry and individually weighed and monitored for ^{131}I and ^{125}I.

Fig. 2. Accumulation of various injected radio-iodinated proteins by laying hen oocytes. Each experiment was performed by intravenous co-injection into a laying hen, of a pair of radio iodoproteins. At the time indicated, the bird was exsanguinated, the ovary removed, and the oocytes treated as indicated for Fig. 1. The ordinate scales are in arbitrary units. Hc, hemocyanin; γ-Lv, γ-livetin; Con, conalbumin; Pv, phosvitin.

oocytes. Thus, without isolating the proteins in each experiment, we were able to obtain a measure of the relative uptake of the tagged proteins to determine if they were being sequestered at the same or different relative rates during oocyte growth. In the larger cells, the rates of accumulation of each protein manifest parallel changes with oocyte weight increment. However, in oocytes less than 200 mg, while the rate of phosvitin accumulation also increases progressively with oocyte size, the specific rate of IgG accumulation (^{131}I cpm/g oocyte wet weight) remains constant. In the upper part of Fig. 1, these results are plotted as a ratio of the two tracer isotopes in oocytes of different weights. The change in IgG accumulation is then apparent as a sharp break in the curve at an oocyte weight of 200 mg. Thus, at a developmental stage in the oocyte corresponding to 200 mg wet weight, there is a discontinuity in the capacity to accumulate IgG when compared with phosvitin.

Further results for some different proteins and varying pulse periods are recorded in Fig. 2 as the ratio of the accumulated isotopes. Varying the interval between injection and sacrifice does not alter the nature of the above described change (Figs 2E and 2D). Substitution of γ-livetin (the yolk homologue of IgG) for IgG is without effect on the results (Figs 2C and 2D). At least two other proteins can substitute for phosvitin without modifying the transition for IgC accumulation, *viz.* conalbumin, a serum transfering homologue, purified from egg white and charged with iron (Fig. 2B); and the heterologous protein, keyhole limpet hemocyanin (Fig. 2A).

Table I records the weight concentrations of phosvitin and γ-globulin in laying hen serum and the yolk of freshly laid eggs. When compared to serum, phosvitin is 6-fold more concentrated in yolk, while IgG is only 0.6-fold as concentrated. That is to say, the ratio of phosvitin to IgG is ten times greater in the yolk than is the ration of the same proteins in serum. This suggests that for the largest oocytes (which most closely approach the composition of mature egg yolk) the rate of accumulation of phosvitin is ten times greater than that of IgG. Since the larger oocytes are responsible for the preponderance of the oocyte protein accumulation[1], the depletion of serum phosvitin should thus be 10 times faster than that of IgG, assuming that the ovary is the principal sink for these serum proteins.

TABLE I

CONCENTRATION OF PHOSVITIN AND γ-GLOBULINS IN SERUM AND YOLK

	Phosvitin concn (g/100 ml)	*γ-Globulin concn (g/100 ml)*	*Ratio: phosvitin concn/ γ-globulin concn*
Serum	0.3	2.7 (IgG)	0.11
Yolk	1.75	1.5 (γ-livetin)	1.17
Concentration factor (yolk/serum)	6×	0.6×	≈10×

In three independent determinations of the serum half lives of iodine-labelled phosvitin and IgG in the laying hen, the following ranges were obtained: phosvitin, 1–2 h; IgG, 10–20 h. Labelled phosvitin is thus being cleared ten times faster than is labelled IgG from the circulation. This value for the relative clearance of the two

iodinated proteins, agreeing so well with that expected, suggests that the isotope-labelled proteins are not sufficiently modified from the native state to be differentially removed by the reticulo-endothelial system. This contention is further born out by the observation that radioactivity in samples of other tissue from an experimental hen was always low (on a per weight basis) when compared with the larger oocytes from the same bird. Also, trichloroacetic acid precipitation of serum indicated 2% or less nonprotein-bound radioactive iodine at the termination of an experiment.

Although phosvitin is found as a free molecule in yolk, within laying hen plasma it exists as a lipophosphoprotein complex, being tightly linked to lipovitellin[10]. Thus, the above indicated agreement of observed rate of relative clearance with that expected, also implies that determinants for ovarial accumulation and/or transport of the serum lipophosphoprotein complex, are identical. That is to say, all determinants for transport and/or accumulation of serum lipophosphoprotein complex must be on the phosvitin moiety. Alternatively, the small amount of radio-iodophosvitin injected into the laying hen circulation is rapidly exchangeable with phosvitin in the lipophosphoprotein complex, and the observed half life is in fact the half life of that complex within the circulation.

In summary, these results give evidence for a sharp change in the capability of the developing oocyte to accumulate IgG when compared with some other proteins that are sequestered. This change is complete at approximately the 200-mg stage in the development of the oocyte.

We believe that the results are explicable in terms of a limited number of alternatives. The principal of these are: (1) There is a change in the selective potential of the oocyte plasma membrane for recognition and transport of IgG during the early phases of oocyte development. Initially, this potential is low, but becomes fully developed at about the 200-mg stage. (2) There is a change in internal selection, culminating at the 200-mg stage. Prior to 200 mg, the endocytosed IgG is either directly re-extruded or degraded and the breakdown products eliminated.

These alternatives, *viz.* (1) surface selection and (2) internal selection are not mutually exclusive. However, from a teleological viewpoint, alternative (2) is a biologically wasteful process. There is, in addition, good evidence in other systems to suggest that alternative (1) is at least in part responsible for the observed changes. Thus, in several systems known to transport or accumulate γ-globulins, the Fc fragments[11] of the immunoglobulin bears a membrane recognition region[12,13]. Moreover, in earlier work, one of the present authors was able to correlate membrane micro-pinocytotic activity with protein accumulation by mosquito oocytes[14]. In the present system, preliminary electron microscopic observations show large number of coated pits and vesicles on and below the oocyte plasma membrane.

It is hoped that further work in progress, employing ultra-structural methods and electron-dense conjugates, will delimit the significance of the proposed two alternatives for an explanation of the phenomena recorded in this paper.

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