sum of volumes for the 7 organs studied) for the cooled group remains the same and total visceral cell volume increases slightly; however, the only statistically significant difference is the increase in cell volume of the spleen. The change in venous hematocrit could be explained if there were a "trapping" or sequestering of red cells by the spleen and liver, and to some extent by a fall in total muscle plasma volume. Total residual plasma volume, however, decreases from an initial volume of 16.8 cc to a level of 6.71 cc/1000 g in cooled alligators. Other changes must be postulated in addition to the cell volume changes in spleen and liver to explain the magnitude of the fall in venous hematocrit; possibly the "lost" plasma of the muscles is added to the venous circulation.

Rapid warming of alligators to 35°C, approximately 10°C higher than the temperature to which they had been acclimated, caused no significant change in venous hematocrit (22.9 ± 0.52) although there was an upward trend in the hematocrits. There was a significant increase in total plasma volume to 72.8 ± 3.77 cc/1000 g but cell volume remained constant. As a result, the circulatory hematocrit fell to 14.75 and the volume residual plasma increased to 30.5/1000 g. Significant increases in organ plasma volumes were noted in the liver, lungs, intestine, kidney, and skin. Significant decreases in cell volumes were noted for intestine and kidneys, and, in addition, total visceral cell volume fell. Thus, total visceral plasma volume increased, while total visceral cell volume decreased. The venous hematocrit, therefore, may increase sightly in a majority of normal animals in spite of widespread increases in plasma volume because of release of red cells from viscera, particularly the intestines and kidneys. At the same time circulatory hematocrit falls because of the increases in plasma volume presumably coming from fluid released by extra-circulatory sources into the small vessels of the viscera, skin and possibly the muscles, but more specifically into the liver, lungs, intestines, kidneys, and skin.

Summary. Cooling the alligator rapidly to 5°C results in a lowered venous hematocrit but does not cause significant change in circulatory hematocrit. Total visceral cell volume tends to increase, although of the individual organs only one, the spleen, has a statistically significant increase in cell volume. Rapid warming of alligators to temperature of 35°C results in no significant change in venous hematocrit but in a significant fall in circulatory hematocrit. Organ cell volumes in general are lower, that of the intestine and kidneys significantly so. This release of cells must account for the maintenance of a constant venous hematocrit in spite of a great increase in plasma volume not accounted for solely by the significant increases in organ plasma volumes.

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Received July 27, 1961. P.S.E.B.M., 1961, v108.

Antigenic Properties of Liver Cell Fractions Derived from Laying Hens and Estrogenized Chickens. (26901)

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It is known that there are phosphoproteins in the sera of laying hens and estrogenized chickens, but not in the sera of non-laying hens, cocks and immature chickens, and that they are produced in the liver by the effects of estrogens(1,2,3,4). The fluctation of

phosphoprotein level and of its properties in the sera of laying hens has been studied serologically, chemically and isotopically (5,6,7,8).

Recently, serum albumin and globulin syntheses *in vitro* have been performed by liver slices. Peter *et al.*(9) developed an immunological method by which the small amounts of serum albumin present in liver slices could be determined and showed that the liver was locus of serum albumin production. Higashi (10) also studied the production of serum globulin *in vitro,* using liver slices and their homogenates. There is no known report on phosphoprotein synthesis *in vitro* by chicken liver slices. Direct demonstration of protein syntheses *in vitro* is complicated by the fact that total protein content of tissue slices decreases upon incubation, owing to the predominance of proteolytic reactions. Our experiments are intended to clarify the mechanism of phosphoprotein synthesis in liver cells of the chicken, and to study the antigenic properties of liver cell fractions of laying hens and estrogenized immature chickens.

Materials and methods. Chickens used for fractionations of liver cell components were White Leghorn laying hens, cocks, and 30-day-old chickens injected with 0.625 mg of diethylstilbestrol intramuscularly every day for 5 days. After serologically demonstrating the presence of, and determining level of phosphoproteins in their sera, the chickens were sacrificed by bleeding in a cool room at 4°C. The liver was perfused with 0.15 M NaCl solution and removed for fractionation of liver cell components. The method of Hogeboom(11) was used for fractionation. Ten ml of liver tissue was sliced, washed with 0.15 M NaCl and homogenized (20% homogenate with 0.25 M sucrose solution). Nuclear, mitochondrial and microsomal fractions were washed by centrifugation twice with 0.34 M sucrose solution and once with distilled water. Each fraction was suspended in 10 ml of distilled water and used for immunization. A similar fractionation technic was used to get spleen cell components from estrogenized chickens. Fractions of spleen cells and of cock sera were dried by freeze-drying before using them in the absorption of antibodies, subsequently called DCS (Dried Cock Serum). The supernatant fraction was diluted with 10 volumes of distilled water and kept at room temperature for 2 days. The precipitate thus obtained was used for absorption. Immune sera were obtained by injecting rabbits intravenously with 2 ml of each suspension every other day 4 to 5 times, and 7 days after the last injection, animals were bled from their jugular arteries. Antisera were inactivated by keeping them in a water bath at 56°C for 30 minutes and 0.1% by volume of a Na-azide solution was added. S-I and S-III fractions of laying hen serum were prepared by the ultracentrifugation technic described by Tanabe *et al.*(12). The technic of interfacial precipitin reaction was described earlier(4). The following technic (Oudin) was used for the agar gel diffusion reaction: Antiserum was mixed with the same volume of 1% agar gel solution at 56°C and poured at once into test tubes of 3 mm diameter to a height of about 2 cm. After coagulation of the agar gel, about the same volume of test serum was layered on the gel, then covered with liquid paraffin to prevent evaporation of the test serum. The test tubes were kept at room temperature (10-18°C) for 4 days. The reactions were read by naked eye. Since 60 mg of DCS was equivalent to a net weight of 1 ml cock serum, 90 mg of DCS were added to each ml of immune serum to exhaust the species specific antibodies. Besides the antisera shown in Table I, all antisera used were absorbed with DCS to exhaust the species specific antibodies. Ten mg of a microsome fraction prepared by freeze-drying from an equivalent of net weight of 5 g of liver tissue, or precipitates taken from 15 ml of supernatant fraction, were added to 0.5 ml of antisera to test the absorbing properties of their antibodies. Titration of antibodies was carried out by the precipitin reaction, serially diluting antisera with 10% NaCl solution.

Results. The results of serological precipitin reactions obtained by antisera against liver and spleen cell fractions of laying hens are shown in Table I.

All unabsorbed antisera showed positive reactions to sera of laying hens and cocks.

TABLE I. Precipitin Reactions of Antisera Obtained with Liver and Spleen Cell Fractions of Laying Hens.

Antisera obtained with fractions of	Antigen	Antisera: Unabsorbed — Dilution of antigen 20	40	80	160	320	640	Antisera: Absorbed with DCS — Dilution of antigen 20	40	80	160	320
Liver nucleus	Cock	3	2	1	0	0	0	0	0	0	0	0
	Hen	3	3	3	3	2	0	0	0	0	0	0
Mitochondria	Cock	2	2	0	0	0	0	0	0	0	0	0
	Hen	3	3	3	3	1	0	0	0	0	0	0
Microsome	Cock	3	3	2	2	0	0	0	0	0	0	0
	Hen	3	3	3	3	3	3	3	3	3	3	1
Supernatant	Cock	3	3	3	3	0	0	0	0	0	0	0
	Hen	3	3	3	3	3	3	2	2	2	1	0
Spleen microsome	Cock	2	2	2	2	2	0	0	0	0	0	0
	Hen	2	2	2	2	1	1	0	0	0	0	0
Supernatant	Cock	3	3	3	3	2	2	0	0	0	0	0
	Hen	3	3	3	3	1	1	0	0	0	0	0

3, 2 and 1 show the appearance of positive reaction within 15, 30 and 60 min., respectively; 0 shows no reaction.
DCS = Dried cock serum.

After absorption of antisera with DCS, each antiserum against the microsomal and supernatant fractions of laying hens showed good reactions to laying hen serum, but not to cock serum. However, antisera against microsomal and supernatant fractions of spleen cells and nuclear and mitochondrial fractions of liver cells from laying hens did not show any positive reaction for presence of antigenic properties of phosphoprotein. No antisera were produced against phosphoproteins in sera of laying hens by liver cell fractions of cocks. These results were confirmed in 3 experiments with microsomal and supernatant fractions of laying hens.

Similar experiments were carried out to test for presence or absence of antigenic properties of phosphoprotein in microsomal and supernatant fractions from liver cells of estrogenized or normal immature chickens. The results were similar to those shown for the antigenic properties of microsomal and supernatant fractions of estrogenized immature chickens. No reactions were produced by other fractions from the same animals and fractions of liver cells of untreated immature chickens.

Table II summarizes absorption tests of anti-Mc(lay) and anti-Sup(lay) with S-I and S-III fractions of laying hens, respectively.

Before absorption of antisera, they showed positive reactions with both fractions of S-I and S-III. After absorbing anti-Mc(lay) with S-I, however, the antiserum showed no reaction with S-I, but reacted positively with

TABLE II. Absorption Tests of Antisera with S-I and S-III Fractions of Laying Hens.

Antisera	Absorbed with	Dilution of antigen: S-I 20	40	80	160	320	640	S-III 20	40	80	160	320	640
Anti-Mc(lay)	Unabsorbed	1	1	1	1	0	0	3	3	3	2	2	0
	S-I	0	0	0	0	0	0	3	3	3	1	0	0
	S-III	0	0	0	0	0	0	0	0	0	0	0	0
Anti-Sup(lay)	Unabsorbed	3	3	3	3	2	0	3	3	3	3	3	1
	S-I	0	0	0	0	0	0	3	3	3	3	1	0
	S-III	3	2	1	1	0	0	0	0	0	0	0	0

Mc(lay) and Sup(lay) are microsome and supernatant fractions derived from liver cells of laying hens, respectively.

TABLE III. Decreases of Antibody Titers by Absorption with Microsome and Supernatant Fractions.

Dilution of antisera	Antigen	Anti-Mc(lay) Unabsorbed — Dilution of antigen					Anti-Mc(lay) Absorbed with Mc(lay) — Dilution of antigen					Anti-Mc(lay) Absorbed with Mc(cock) — Dilution of antigen					Anti-Mc(lay) Absorbed with Mc(lay-spleen) — Dilution of antigen				
		20	40	80	160	320	20	40	80	160	320	20	40	80	160	320	20	40	80	160	320
1	S-III	3	3	3	3	2	0	1	1	1	0	3	3	3	3	2	3	3	3	3	2
2		3	3	3	3	2	0	0	0	0	0	3	3	3	3	2	3	3	3	3	2
4		1	2	2	1	1	0	0	0	0	0	1	2	2	1	0	1	2	2	1	0
8		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Anti-Sup(lay) Unabsorbed					Anti-Sup(lay) Absorbed with Sup(lay)					Anti-Sup(lay) Absorbed with Sup(cock)					Anti-Sup(lay) Absorbed with Sup(lay-spleen)				
1	S-I	3	3	3	3	2	0	0	0	0	0	3	3	3	3	2	3	3	3	3	2
2		3	3	3	3	2	0	0	0	0	0	3	3	3	3	2	3	3	3	3	2
4		3	2	2	2	0	0	0	0	0	0	2	2	1	1	0	3	2	2	1	0
8		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Mc(lay-spleen) and Sup(lay-spleen) are microsome and supernatant fractions derived from spleen cells of laying hens, respectively.

S-III. Absorption of the antiserum with S-III fraction exhausted antibodies to S-I and S-III fractions. Absorption tests of anti-Sup(lay) with S-I and S-III fractions showed that S-I and S-III fractions removed antibodies for themselves from the antiserum, but did not remove antibodies for other fractions.

In the following experiment, titration of antibodies in anti-Mc(lay) and anti-Sup (lay) absorbed with microsomal and supernatant fractions of liver and spleen cells of laying hens and of liver cells of cocks, respectively, was carried out (Table III).

Antibody to S-III in the anti-Mc(lay) was almost removed by absorption with Mc(lay). But no decrease of antibody occurred by absorption with Mc(cock) and Mc(lay-spleen), respectively. Similarly, antibody to S-I fraction in the anti-Sup(lay) was exhausted by absorption with Sup(lay), but not by absorption with Sup(cock) and Sup(lay-spleen).

Antisera to each fraction of liver cells of laying hens were used for agar gel diffusion reaction with sera of laying hens and cocks and with S-I and S-III fractions. In these experiments, one positive zone reaction appeared in the agar gel of anti-Mc(lay) with serum and S-III fraction of laying hens and 2 reactions in the agar gel of anti-Sup(lay) with serum and S-I fraction of laying hen. Absence of positive zone reaction to S-I fraction of laying hen in the agar gel of anti-Mc(lay) is assumed to be due to a deficiency of antibody against the S-I fraction of laying hen. Thus, the results obtained here by agar gel diffusion reaction demonstrated again the presence of the specific antigenic properties of S-I and S-III fractions of laying hen serum in microsomal and supernatant fractions of liver cells of laying hens and estrogenized immature chickens.

Discussion. It has been known that the liver is the locus of serum albumin and globulin production in mammals and that the microsomal fraction plays an important role in protein synthesis in liver cells(13,14). In our experiments, microsomal and supernatant fractions of liver cells of laying hens and of estrogenized chickens showed immunologically antigenic properties equivalent to phosphoproteins present in the sera of laying hens. However, fractions of liver cells of cocks and immature chickens and of spleen cells of estrogenized chickens had no such antigenic properties. The absence of specific antibodies in the antisera produced by immunizing with nuclear and mitochondrial fractions of liver cells and microsomal and supernatant fractions of spleen cells derived from laying hens and estrogenized chickens suggests that the antigenic properties of phosphoproteins found

in the 2 fractions of liver cells of laying hens were not those induced by residual serum proteins in liver homogenates. The antigenic properties of phosphoproteins found in microsomal and supernatant fractions of laying hens and estrogenized chickens appear to be specific for the 2 fractions of their liver cells. These findings give immunological support to the idea that the liver is the locus of phosphoprotein production in the laying hen. Microsomal and supernatant fractions of liver cells of laying hens had both specific antigenic properties of S-I and S-III, in which antigenicity of S-I in the microsomal fraction was weak. The reason for this weakness is not clear. The results permit the assumption that microsomes play an important role in phosphoprotein syntheses in liver cells or that they produce the S-I fraction (low density lipoprotein) and S-III fraction (phosphoprotein) in liver cells of laying hens and secrete them into blood stream.

Summary. Microsomal and supernatant fractions of liver cells of laying hens and estrogenized chickens showed immunologically specific antigenic properties similar to those of phosphoproteins found in the sera of laying hens. However, nuclear and mitochondrial fractions derived from liver cells of laying hens, 4 fractions from spleen cells of laying hens and 4 fractions from liver cells of cocks, did not show any such specific antigenic properties. Microsomal and supernatant fractions of liver cells of laying hens and estrogenized chickens had both specific properties of S-I and S-III fractions. Microsomal and supernatant fractions absorbed well anti-S-III and anti-S-I antibodies, respectively.

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Received July 6, 1961. P.S.E.B.M., 1961, v108.

Elevation of Proteolytic Activity in the Pancreas of Hypophysectomized Rats by Hormonal Therapy.* (26902)

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Following hypophysectomy a severe reduction in weight of the pancreas occurs in the rat(1) and dog(2). In the rat most acini are partially involuted with loss of cellular size, zymogenic granules and cytoplasmic RNA(3,4,5). Concurrently, the amount of amylase(6) and proteolytic activity(7) is reduced. Experiments aimed at clarifying the hormonal pathway by which the hypophysis exerts its influence over the pancreas have entailed both ablation of other endocrine glands and hormonal replacement therapy of endocrine-deficient animals. These studies reveal that thyroid hormone plays a key role(3,8,9,10,11,12) in maintaining the

* Supported in part by research grants from U.S.P.H.S., Inst. Res. Grant, Am. Cancer Soc., and Upjohn Co.