dissolved in water. Doses of 6 or 8 mg INH, lethal *per se* in aqueous solution, gave 50% mortality after 7 intermittent (twice weekly) administrations when 35% or 50% glycerine solution respectively was employed as solvent. With larger amounts of INH the requisite amount of glycerine lay in the toxic range. The absorption curves indicate that significantly higher blood plasma levels of INH were obtained with the glycerine solutions than with aqueous solutions. In higher concentrations of glycerine there was apparently synergism with INH in the *in vitro* bacteriostatic action on 2 human strains of *Mycobacterium tuberculosis.*

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Formation of Porphyrin from Delta-Aminolevulenic Acid by Uterine and Liver Tissue from Laying Hens.* (22919)

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A variety of avian tissues and organs are capable of forming porphyrins, *in vitro,* from delta-aminolevulenic acid (ALA): namely, chicken erythrocytes(1,2), duck erythrocytes (2,3), pigeon liver and breast muscle(4). The uterus of the hen has not been similarly characterized, and would be a likely organ to form porphyrin from ALA, *in vitro,* as oöporphyrin is deposited on the egg shell within this organ.

Methods and materials. White Leghorns, which lay white eggs, and Crossbreeds and New Hampshires, which lay brown eggs of varying shade, were used in these experiments. At a time when no egg was in the uterus, birds were killed by decapitation and uterus, portions of liver and of breast muscle were removed immediately and placed on crushed ice. In a cold room (6°C), 1 g tissue was homogenized in a mortar containing coarse sand and 4 successive 5 ml aliquots of buffered saline solution, pH 7.2(5). After each grinding supernatant solution was decanted into a 25 ml graduate, and finally contents of the mortar were rinsed into the graduate with buffered-saline and diluted to 25 ml. The graduate was stoppered, the contents mixed and allowed to stand in crushed ice for 5-10 minutes. Aliquots of 0.2, 0.4, 0.8 and 1.6 ml (equivalent to 8, 16, 32 and 64 mg of tissue) from the upper half of the graduate were pipetted into tubes containing 1.4 ml of 0.008 M ALA in buffered-saline. One tube containing 1.6 ml of solution but no ALA, and another tube without the solution but with ALA were used as controls. All tubes were made up to 4.4 ml with buffered-saline, capped and incubated at 41°C for 24 hours. Each series of tubes was run in duplicate.

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After incubation, 5 ml of concentrated HCl were added to each tube. After 2 hours the contents were transferred to 15 ml conical centrifuge tubes with washings of buffered-saline. The volumes were made up to 12 cc with buffered-saline, and the tubes centrifuged. An aliquot of each tube was diluted with 3N HCl and read in the Beckman DU spectrophotometer against a control blank at 408 mμ and also at 382 and 432 mμ to obtain corrected density. Concentration of porphyrin was determined from a standard protoporphyrin curve. In experiments in which porphyrins were characterized, the incubated solution was first extracted with 4:1 ethyl acetate-glacial acetic acid until no further fluorescence could be observed in extracts examined under UV light. If porphyrins remained in the precipitate of the incubated solution, they were removed with 3N HCl washings. The acid washings were neutralized with saturated sodium acetate, extracted with ethyl acetate-acetic acid and added to porphyrin already obtained. Porphyrins were fractionated by the procedure of Dresel and Falk(2). Porphyrin was obtained from brown egg shells by dissolving the shells in 3N HCl and filtering the solution through Whatman #1 paper. The acid solution was adjusted to pH 4.8-5.5 with saturated sodium acetate, the oöporphyrin extracted with ethyl acetate-acetic acid, and fractionated(2). Absorption curves were determined on this oöporphyrin and on the porphyrins formed *in vitro* by supernatant solutions prepared from uterine tissues.

Results. Exp. 1. Supernatant preparations of liver and uterine tissues from hens laying brown eggs formed a considerable amount of porphyrins from ALA; whereas muscle preparations produced only trace amounts (Fig. 1). A linear relationship existed between amount of porphyrins formed and amount of tissue used, over the range studied. Regression equations (Fig. 1) were calculated from the data, and it was determined that 100 mg of uterine tissue, wet weight, formed 132 γ of porphyrins as compared to 62.5 γ by liver tissue (expressed in equivalents of protoporphyrin).

Exp. 2. Uterine and liver tissue preparations from hens that laid white eggs formed as much porphyrin as similar tissue from hens that laid brown eggs (Tables I and II). There was considerable variation between hens in porphyrin-forming capacity of these tissues, but for each hen, there was a relatively constant ratio of approximately 2:1 for the amount of porphyrin formed per unit wet weight of uterine tissue as compared to liver. As shown in Table I, this ratio is approximately the same whether computed from ratio of slopes, b_{uterus}/b_{liver} of regression equations or as a ratio of porphyrins formed per 100 mg tissue (obtained by extrapolation of regression equations).

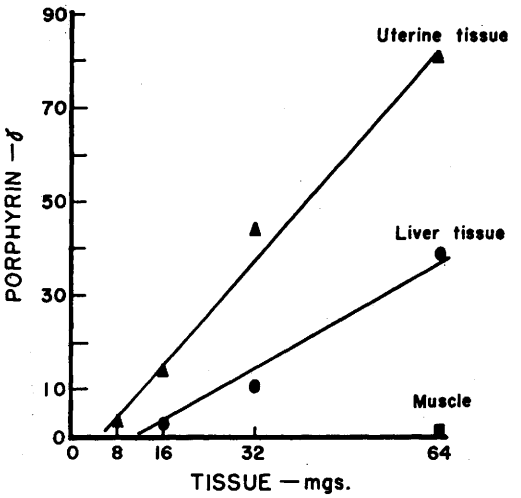

FIG. 1. Amount of porphyrin formed by uterine, liver and muscle tissues, from laying hens, incubated at 41°C for 24 hr in 4.4 ml buffered-saline, pH 7.2, containing .41 mg/ml of delta-aminolevulenic acid. $Y = -5.9 + 1.38X$ (X = mg uterine tissue); $Y = -8.3 + .708X$ (X = mg liver tissue).

TABLE I. Amount of Porphyrin Formed by Supernatant Preparations of Uterine and Liver Tissue from Hens Laying Brown or White Eggs.

Bird type and No.		γ porphyrins/100 mg tissue, wet wt—calculated from regression equations			Ratio of regression slopes
		Glandular tissue of uterus	Liver	Uterus / Liver	b_{uterus}/b_{liver}
Brown egg layer	1	132.8	62.5	2.12	1.97
	2	101.7	55.0	1.85	1.77
	3	105.2	53.8	1.96	1.88
White egg layer	1	116.8	61.6	1.90	1.74
	2	305.8	145.2	2.11	2.31

TABLE II. Porphyrin Forming Capacity of Supernatant Preparations of Uterine Tissue from Hens Laying Either a White, Tinted, or Brown Egg.

Brown egg layers		Tinted brown egg layers		White egg layers	
Breed	Porphyrin, γ/100 mg uterine tissue	Breed	Porphyrin, γ/100 mg uterine tissue	Breed	Porphyrin, γ/100 mg uterine tissue
New Hampshire	138.2	New Hampshire	105.2	White Leghorn	99.6
" "	132.8	Crossbreed	175.3	" "	429.0
" "	101.7	White Plymouth Rock	258.5	" "	260.8
" "	192.8	" " "	271.9	" "	214.0

Exp. 3. Porphyrins formed *in vitro,* had an absorption curve similar to that of oöporphyrin (Fig. 2), but with maximum peak at 407-408 mμ and another at 480 mμ. These porphyrins could be fractionated into 3 components tentatively identified as uro-, copro- and protoporphyrin. Uroporphyrin had an absorption peak at 405-407 mμ in 0.6N HCl, and comprised 91.4 ± 1.4% (mean ± S.E.M.) of porphyrins extracted. Coproporphyrin, with absorption peak at 401 mμ in .12N HCl, was present in trace amounts (.5 ± .18%), and protoporphyrin, with absorption peak at 407-408 mμ in 3N HCl accounted for 9.7 ± 0.98%. These results are based upon analyses of tissue from 7 different hens.

Two to 3 g samples of uterine glandular tissue, from hens that laid brown eggs, were extracted for porphyrins, in the dark for 18 hours. The amount of porphyrins found in 5 different uteri ranged from 1.1 to 15.5 γ/g (mean = 6.9 γ/g) of uterine tissue. Partition of the porphyrins showed that a protoporphyrin-type compound (with the same characteristics as shell porphyrin) comprised 97.1% of the total; whereas coproporphyrin accounted for the remainder.

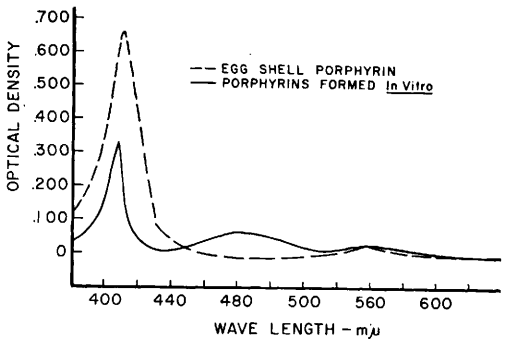

FIG. 2. Absorption spectra of porphyrin from egg shell (max. 410 mμ) and porphyrins formed by uterine tissue supernatants (max. 408 mμ), both in 3N HCl. Measurements were made with a Beckman DU spectrophotometer. Cell thickness, 1 cm.

Discussion. Investigators have suggested that shell porphyrins on the hen's egg are derived by hemoglobin degradation when erythrocytes are ruptured in the uterus, and pigment masses excreted onto shell(6). Although hemoglobin degradation to porphyrin cannot be excluded as a source for shell protoporphyrin, this pathway has not been proved. In fact, data indicate that hemoglobin degradates to bile pigments, *in vivo* (7), while protoporphyrin is formed via heme synthesis(8,9).

Porphyrins are synthesized in a cycle of which ALA is an intermediate(8,9). Uterine tissue contains considerable capacity for transforming ALA to porphyrins, *in vitro,* and it is suggested this tissue forms shell porphyrins via this cycle. The avian red cell can form protoporphyrin from ALA both *in vitro* (1,3) and *in vivo*(8,9), and may possibly contribute to the shell porphyrin pool in this manner, rather than by degradation of hemoglobin as suggested(7).

Most interesting is the fact that uterine tissue of a hen that laid a white egg, whose shell contained less than 10 γ of oöporphyrin, forms as much porphyrin, *in vitro,* from ALA as uterine tissue from a hen that laid a brown egg, the shell of which contained as much as 100 to 350 γ oöporphyrin. There is a possibility that ALA, or a precursor to ALA, is limiting in uterine tissue of the hen that lays a white egg, and this may account for the very small amount of oöporphyrin on the shell.

Sample preparations and substrate concentrations(2), in addition to other unknown factors, may account for dissimilarity in type

of porphyrins formed *in vitro* as compared to those formed *in vivo*. Uroporphyrin-like compounds are formed predominately by preparations of uterine tissue; whereas, almost all porphyrin deposited on shell and found in intact uterine tissue is a protoporphyrin-type compound. Some coproporphyrin, 0.2 γ/g of uterine tissue, is also found.

Summary. 1. Uterine tissue, from white or brown egg-laying hens, forms twice as much porphyrin, *in vitro,* from delta-aminolevulenic acid as liver tissue. Supernatant preparations of uterine tissue form predominately uroporphyrin, small amounts of protoporphyrin, and trace amounts of coproporphyrin from delta-aminolevulenic acid. These compounds were tentatively identified by their behavior in the fractionation procedure used and by their absorption curves. 2. Oöporphyrin and coproporphyrin were extracted from the glandular tissue of the uterus of laying hens.

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Role of Lipid in Hemagglutination by *Candida albicans* and *Saccharomyces cerevisiae.** (22920)

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An erythrocyte sensitizing antigen (ESS) from saline washings of bead-disrupted *Candida albicans* and *Saccharomyces cerevisiae* has recently been described(1). The activity of the antigen was measured in the hemagglutination reaction in which simple admixture of erythrocytes with polysaccharides leads to acquisition of immunologic activity by the red cells as demonstrated by their agglutination in homologous antiserum. In addition to such activity these preparations were noted to possess immunologic precipitating properties while on the basis of positive Molisch tests and negative Biuret tests they were characterized essentially as polysaccharide. Recent observations have shown that occasionally the erythrocyte sensitizing activity of an antigen may disappear spontaneously on standing while its precipitating activity remains unchanged.

In the experiments reported below it is proposed that saline extracts from these organisms, with the overall properties of polysaccharides, contain lipid which is necessary for the sensitization of red cells. The current interest in the pyrogenic, endotoxic and hemagglutinative properties of the lipopolysaccharides from Gram-negative bacteria(2,3) makes the findings reported below of antigenic compounds believed to be lipopolysaccharide isolated from yeast and yeast-like organisms of considerable interest.

Materials and methods. Antigen prepara-

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Pancreatic extract, lot # 8279-3 was kindly supplied by Dr. Kenneth W. Thompson, Medical Director, Organon Inc.