Alteration of Egg Yolk Cholesterol Content and Plasma Lipoprotein Profiles following Administration of a Totally Synthetic HMG-CoA Reductase Inhibitor to Laying Hens[†]

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PD 123244-15 [(±)-(R^*,R^*)-3,4-dibromo-β,δ-dihydroxy-2-(4-fluorophenyl)-5-(1-methylethyl)-$1H$-pyrrol-1-heptanoic acid, sodium salt], a totally synthetic HMG-CoA reductase inhibitor, was fed to laying hens at dietary levels of either 0, 0.0091, 0.0273, or 0.0819% for 20 days (experiment 1) or 0, 0.0273, 0.0546, or 0.0819% for 42 days (experiment 2). In each experiment, PD 123244-15 generally had little effect on egg or yolk weights, while hens fed 0.0819% PD 123244-15 produced fewer ($P < 0.05$) eggs than the other three groups. Egg cholesterol contents varied inversely with the amount of dietary PD 123244-15, with a maximum reduction of approximately 30% obtained at the highest drug level in both experiments. With the exception of samples from hens fed 0.0091% PD 123244-15, plasma total cholesterol and triglyceride concentrations also varied inversely with the dietary amount of PD 123244-15. Further analysis of the plasma samples by high-performance gel permeation chromatography revealed that the PD 123244-15-mediated lowering of total cholesterol and triglycerides was almost totally accounted for by a reduction in the VLDL fraction. On the basis of reversed-phase HPLC analysis, no drug residues were detected in egg yolk and albumen extracts from hens fed 0.0819% PD 123244-15.

INTRODUCTION

The association of total plasma cholesterol levels with the incidence of coronary heart disease (CHD) is well established (Lipid Research Clinics Program, 1984; National Institutes of Health Consensus Development Panel, 1985). However, the role of dietary cholesterol as a contributor to hypercholesterolemia and atherogenesis in humans is a topic of considerable scientific research and debate (McNamara, 1990). Nevertheless, restriction of dietary cholesterol to less than 300 mg/day has been recommended for all Americans (National Institutes of Health Consensus Development Panel, 1985). Although some researchers feel that limitation of cholesterol intake for the population as a whole is not warranted (McNamara, 1990), others support dietary cholesterol restriction (Gotto, 1991), particularly for the 36% of adult Americans who are at high risk for CHD because they are hypercholesterolemic (Sempos et al., 1989).

Irrespective of this controversy, growing public concern over dietary cholesterol is reflected in U.S. annual per capita egg consumption figures, which have declined from 303 to 235 during the past 20 years (U.S. Department of Agriculture, 1987, 1991). Although not readily quantifiable, a portion of this downturn may be attributed to dietary therapy programs (Expert Panel, 1988) for patients with high or borderline-high risk for CHD, in which the consumption of egg yolks, which are a rich source of cholesterol, is severely limited.

From a nutritional standpoint, eggs are an excellent source of high-quality protein, mono- and polyunsaturated fatty acids, several minerals, and numerous fat- and water-soluble vitamins (Cook and Briggs, 1977). Thus, a major reduction in the cholesterol content of eggs would yield a foodstuff of extremely high nutritional quality that could be freely consumed by all segments of the population. Unfortunately, attempts to reduce egg yolk cholesterol levels by altering the laying hens' diet, or through genetic selection programs, have been only marginally successful (Hargis, 1988). It has been suggested that the relative resistance of egg composition to alterations in diet apparently reflects the nutritional and structural requirements for avian embryonic development (Kuksis, 1992).

An alternate approach to lowering egg cholesterol content lies in the administration of pharmacological agents to laying hens. During the past 25 years, many drugs that have been employed as hypocholesterolemic agents in humans have been orally administered to chickens (Naber, 1983; Hargis, 1988). Despite this intense research effort, a compound that greatly reduced egg yolk cholesterol, without causing either a cessation of egg production or an accumulation of undesirable metabolites in the egg, remained elusive.

Recent work in our laboratory with the human hypocholesterolemic drug lovastatin (Elkin and Rogler, 1990) suggested that HMG-CoA reductase inhibitors hold promise as effective egg cholesterol-lowering agents. In the present paper, PD 123244-15 [(±)-(R^*,R^*)-3,4-dibromo-β,δ-dihydroxy-2-(4-fluorophenyl)-5-(1-methylethyl)-$1H$-pyrrol-1-heptanoic acid, sodium salt], a novel, totally synthetic HMG-CoA reductase inhibitor (Krause and Newton, 1991), was evaluated in this regard and shown to markedly reduce egg yolk cholesterol content. In addition, plasma lipoprotein profiles suggested that PD 123244-15 impaired the hepatic synthesis of VLDL, the prevalent lipid carrying plasma protein in mature hens (George et al., 1987) and one of the main components of egg yolk (Nimpf and Schneider, 1991). A preliminary report of this work has appeared (Elkin et al., 1992).

Reduction of Egg Cholesterol and Plasma VLDL by PD 123244-15

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Table I. Effect of PD 123244-15 on Laying Hen Performance (Experiment 1)[a]

diet	PD 123244-15, %	hen–day egg production, %	egg wt, g	yolk wt, g	body wt gain, g	daily feed consumption, g/hen
1	0	92.0 ± 3.4[a]	62.7 ± 0.4[a]	17.6 ± 0.3[a]	46.5 ± 21.2[a]	107.6 ± 4.9[a]
2	0.0091	86.0 ± 5.1[a]	63.9 ± 0.4[a]	17.7 ± 0.3[a]	43.9 ± 21.0[a]	104.9 ± 4.6[a]
3	0.0273	90.0 ± 4.2[a]	62.5 ± 0.4[a]	17.3 ± 0.3[a]	66.8 ± 16.7[a]	114.7 ± 4.0[a]
4	0.0819	73.8 ± 1.3[b]	60.1 ± 0.6[a]	17.3 ± 0.3[a]	99.5 ± 29.2[a]	110.2 ± 2.8[a]

[a] The duration of the experiment was 20 days. Data are presented as means ± SE. Hen–day egg production, body weight gain, and feed consumption data are the mean values of five, five, five, or four hens fed diets 1, 2, 3, or 4, respectively. Egg weights are the mean values of 92, 86, 90, or 59 eggs from hens fed diets 1, 2, 3, or 4, respectively. Yolk weights are the mean values of 15, 15, 15, or 12 yolks from hens fed diets 1, 2, 3, or 4, respectively. Means within each column with different superscripts are significantly different ($P \leq 0.05$).

Table II. Percentage Distributions of Plasma Lipoprotein Cholesterol and Triglycerides in Laying Hens Fed Various Amounts of PD 123244-15 (Experiment 1)[a]

diet	PD 123244-15, %	cholesterol VLDL, %	IDL, %	LDL, %	HDL, %	triglycerides VLDL, %	IDL, %	LDL, %	HDL, %
1	0	27.9 ± 1.1[a]	33.7 ± 3.0[a]	11.6 ± 0.4[a]	26.8 ± 3.2[a]	53.6 ± 1.2[a]	40.8 ± 2.2[c]	4.6 ± 1.1[a]	1.0 ± 0.1[a]
2	0.0091	17.1 ± 4.4[b]	44.4 ± 2.2[a]	11.7 ± 1.6[a]	26.8 ± 5.4[a]	40.8 ± 3.0[b]	53.0 ± 3.5[b]	4.9 ± 1.7[a]	1.3 ± 0.2[a]
3	0.0273	6.5 ± 1.6[c]	40.2 ± 6.0[a]	12.6 ± 0.8[a]	40.7 ± 6.7[a]	23.9 ± 3.7[c]	69.0 ± 4.1[a]	4.7 ± 1.2[a]	2.4 ± 1.1[a]
4	0.0819	3.4 ± 1.1[c]	47.2 ± 1.4[a]	12.2 ± 1.0[a]	37.2 ± 2.0[a]	16.3 ± 2.1[c]	75.7 ± 1.5[a]	6.1 ± 0.7[a]	1.9 ± 0.4[a]

[a] Data are from the profiles depicted in Figure 3. Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 21. Each value is the mean (±SE) of five, five, five, or four samples from hens fed diets 1, 2, 3, or 4, respectively, from day 1 to day 20. Within a column, means with different superscripts are significantly different ($P \leq 0.05$).

MATERIALS AND METHODS

Animals, Management, and Diets. Two experiments of different duration, utilizing hens of different ages and employing different dietary levels of PD 123244-15, were conducted.

Experiment 1. Twenty 60-week-old White Leghorn hens were placed in individual 30 × 35 × 45 cm slant-back cages in an environmentally controlled room [24 °C and 16 h of light (10 lx) daily] in the Layer Research Unit of the Baker–Purdue Animal Sciences Center. Birds were assigned to one of four dietary treatments on the basis of both egg production and average egg weight during the 10 days immediately prior to the initiation of the experiment. During the preexperimental period, egg weights averaged 65.6, 66.0, 65.9, and 66.0 g for hens in treatments 1–4 (Table I), respectively, while all four groups averaged 94% hen-day egg production. Hen–day egg production was calculated as (100 × number of eggs laid)/(number of hens × days). Five hens each were fed either a corn–soybean meal basal layer ration (Elkin and Rogler, 1990) or the basal ration supplemented, at the expense of starch, with 0.0091, 0.0273, or 0.0819% PD 123244-15 for 20 days. These three dietary levels of PD 123244-15 equated to a daily dosage of approximately 10, 30, and 90 mg/hen, respectively, based on a daily feed intake of 110 g/bird (Elkin and Rogler, 1990). PD 123244-15 was synthesized in the Department of Chemistry, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI. Feed and water were supplied *ad libitum* throughout the experiment. At 5:00 p.m. on day 20, all hens were fasted for 15 h and individual blood samples were obtained by cardiac puncture using heparinized needles and syringes. Following procurement of the blood samples, all hens were fed the basal ration from day 21 to day 42 (withdrawal period). The purpose of the withdrawal period was to investigate whether egg cholesterol levels would rise (return to pre-experimental levels) following removal of PD 123244-15 from the diet.

Experiment 2. Twenty-four 43-week-old White Leghorn hens were placed in individual slant-back cages and housed under identical conditions in the same facility as described for experiment 1. Hens were assigned to one of four dietary treatments on the basis of both egg production and average egg weight during the 9 days immediately prior to the initiation of the experiment. All four groups averaged 90.8% hen–day production during the pre-experimental period. Egg weights averaged 59.9, 59.9, 60.2, and 60.2 g for hens in treatments 1–4 (Table II), respectively. Six hens each were fed either a corn–soybean meal basal layer ration (Elkin and Rogler, 1990) or the basal ration supplemented, at the expense of starch, with 0.0273, 0.0546, or 0.0819% PD 123244-15 for 42 days. Feed and water were supplied *ad libitum* throughout the experiment. At 5:00 p.m. on day 42, all hens were fasted for 15 h and individual blood samples were obtained by cardiac puncture using heparinized needles and syringes. Following procurement of the blood samples, all hens were fed the basal ration from day 43 to day 63 (withdrawal period).

Egg Cholesterol Analyses. One egg from each hen was collected on days 0, 7, 14, 20, and 42 (experiment 1) and days 0, 7, 14, 21, 28, 35, 42, and 63 (experiment 2). The eggs were hard-cooked, and the yolks were separated, weighed, and crumbled. A 1-g sample of each yolk was homogenized with 15 mL of chloroform–methanol (2:1 v/v), sonicated, and filtered as previously described (Elkin and Rogler, 1990). Egg homogenate filtrates were analyzed for cholesterol content by the spectrophotometric method of Rudel and Morris (1973).

Plasma Lipid Analyses. Plasma samples were analyzed for total cholesterol content according to the method of Rudel and Morris (1973). Free glycerol-corrected total plasma triglyceride concentrations were determined using a commercial kit (Triglycerides-GB, Boehringer Mannheim Diagnostics, Indianapolis, IN). Plasma lipoprotein cholesterol and triglyceride distributions were determined by high-performance gel filtration chromatography (HPGC) as described by Kieft et al. (1991) using a 10 × 300 mm Superose 6HR column (Pharmacia LKB, Piscataway, NJ). This method employs an on-line gel filtration system in which the cholesterol and triglyceride contents of the different lipoprotein fractions are determined by enzymatic assays. The postcolumn cholesterol and triglyceride quantitation reagents employed were Single Vial cholesterol high performance reagent (Boehringer Mannheim) and Trigly-Cinet (Sclavo Diagnostics, Wayne, NJ), respectively. The HPGC system was calibrated using human lipoprotein standards prepared by ultracentrifugation and has been shown to be utilizable for several different normolipidemic and dyslipidemic animal models (Kieft et al., 1991). Various peaks in the cholesterol and triglyceride profiles were designated VLDL, IDL, LDL, and HDL for simplicity, although it is recognized that separation of the various lipoprotein classes is determined primarily by size of the particles (Kieft et al., 1991). Plasma lipoprotein cholesterol and triglyceride distribution percentages were calculated by dividing the area under each peak, determined by computer integration, by the total area of all peaks.

PD 123244-15 Residue Analyses. In an attempt to determine if PD 123244-15 was transferred to the egg, individual egg yolk and albumen extracts from hens fed 0.0819% PD 123244-15 in experiment 2 were analyzed by HPLC as described below. Samples from hens fed 0% PD 123244-15 served as baseline controls.

Standard Preparation. Standard solutions containing 100 μg of PD 123244-15/mL of acetonitrile–water (90:10 v/v) were prepared. Immediately prior to use, several milliliters of the standard solution was filtered using a Millex-HV 25-mm filter unit (0.45-μm pore size; Millipore Corp., Bedford, MA). Ten
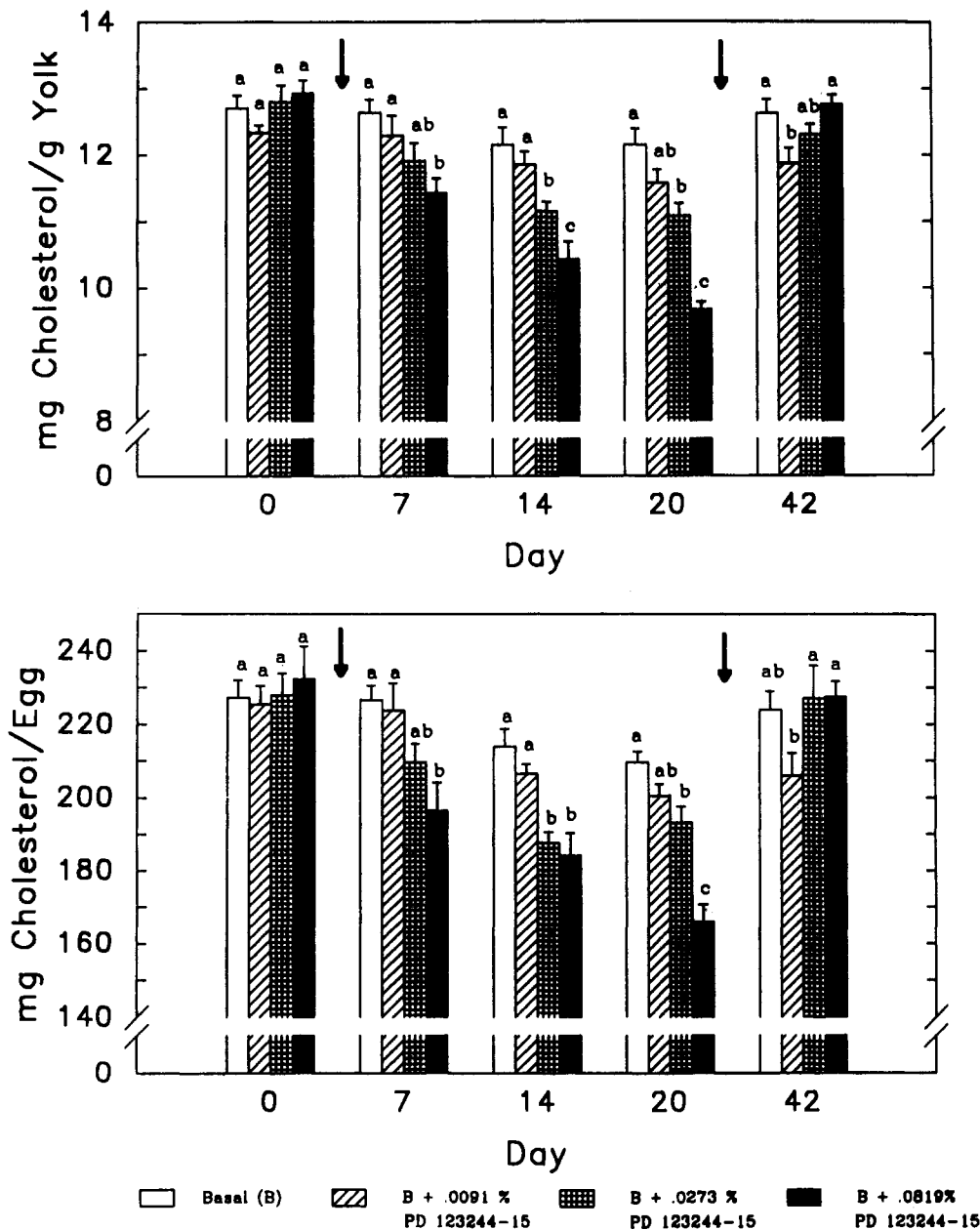
Figure 1. Mean (±SE) cholesterol content, expressed on a per gram of yolk or per egg basis, of eggs from hens fed various amounts of PD 123244-15 (experiment 1). Arrows indicate dietary inclusion (day 1) and withdrawal (day 21) of the drug. Each bar is the mean value of five, five, five, or four eggs from hens fed 0, 0.0091, 0.0273, or 0.0819% PD 123244-15, respectively. Within a day, bars with different letters are significantly different (P < 0.05).

microliters of the filtered solution, which contained 1 μg of PD 123244-15, was subjected to HPLC analysis as subsequently described.

Egg Preparation. Four or nine eggs laid between days 37 and 41 were collected from control hens or hens fed 0.0819% PD 123244-15, respectively. Each egg was weighed and broken, and the yolk and albumen were separated. Any adhering albumen was removed by rolling the yolk on a moist paper towel. To 10 g of yolk or albumen was added 20 mL of acetonitrile–water (90:10 v/v). The samples were sonicated for 60 min, mixing every 15 min, and filtered through a 15-cm Whatman (Hilsboro, OR) No. 1 filter to remove large particulates. Several milliliters of each yolk and albumen extract was then filtered as previously described for the PD 123244-15 standard. Ten microliters of each filtered extract, corresponding to 5 mg of yolk or albumen, was subjected to HPLC analysis as described below.

HPLC Equipment and Chromatographic Procedure. The HPLC system (Waters Associates, Milford, MA) consisted of a Model 6000A pump, a Model 710B automatic sample injector, a Model 720 system controller, a Model 730 data module, a Model 481 variable-wavelength detector (280 nm, 0.02 AUFS), and a Guard-Pak precolumn module containing a μBondapak C18 insert.

The analytical column used was a 4.6 × 250 mm Ultrasphere ODS (5 μm; Beckman Instruments, Fullerton, CA) maintained at 30 °C by using a Model LC 22/23 electronic heater (Bioanalytical Systems, West Lafayette, IN). The mobile phase (flow rate, 1.2 mL/min) consisted of acetonitrile–water–acetic acid (50:49.85:0.15 v/v/v).

Statistics. Analysis of variance was used to statistically analyze all data (Steel and Torrie, 1980). Individual treatment differences were tested by the Duncan multiple range test (Steel and Torrie, 1980).

RESULTS

Experiment 1. PD 123244-15 did not significantly affect egg, yolk, or body weight gains or feed intake (Table I). However, hens fed the highest level of PD 123244-15 produced significantly fewer eggs than the other three groups (Table I). One hen (no. 470) in the 0.0819% PD 123244-15 treatment failed to lay an egg from day 3 to day 20. Although not a common occurrence, cessation of egg production following the transfer of hens to new cages
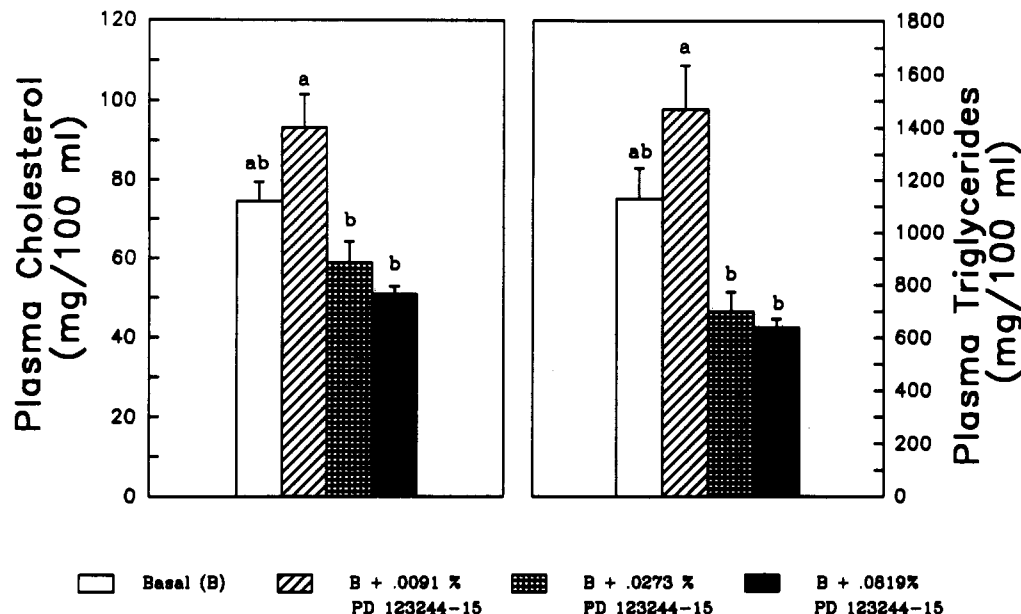
Legend:

□ Basal (B) ▨ B + .0091 % PD 123244-15 ▦ B + .0273 % PD 123244-15 ■ B + .0819% PD 123244-15

Figure 2. Mean (±SE) plasma total cholesterol and triglyceride concentrations of hens fed various amounts of PD 123244-15 (experiment 1). Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 21. Each bar is the mean value of five, five, five, or four samples from hens fed 0, 0.0091, 0.0273, or 0.0819% PD 123244-15, respectively, from day 1 to day 20. Bars with different letters are significantly different ($P < 0.05$).

with different neighboring hens has been previously observed in our laboratory (Elkin and Rogler, 1990) and by other researchers (P. Y. Hester, Purdue University, personal communication). In addition, this phenomenon is more frequently noted in older hens such as the ones used in this experiment. On the basis of this information, the stoppage of egg production in this hen was deemed to be a random event and the bird was eliminated from the study.

Egg cholesterol contents varied inversely with the dietary level of the drug (Figure 1). Although an effect of PD 123244-15 was noted as early as day 7, maximum reductions (vs day 0 values) of approximately 24 and 29% on a per gram of yolk and a per egg basis, respectively, were observed on day 20 in birds fed 0.0819% PD 123244-15. In contrast to those of hens fed 0.0091% PD 123244-15 from day 1 to day 20, cholesterol contents of eggs from hens in the other two drug treatments returned to pre-experimental levels following dietary withdrawal of PD 123244-15 for 22 days (Figure 1).

Plasma total cholesterol and triglyceride concentrations were markedly lower in hens fed the two highest levels of PD 123244-15 vs the other two dietary treatments (Figure 2). Hens fed 0.0819% PD 123244-15 had total cholesterol and triglyceride concentrations that were 68 and 51%, respectively, of those of the control birds. HPGC analyses of the plasma samples revealed that the PD 123244-15-mediated lowering of plasma total cholesterol and triglyceride concentrations was almost totally accounted for by a reduction in the VLDL fraction (elution time approximately 20 min; Figure 3). On a percentage distribution basis (Table II), this resulted in an increase in the amount of cholesterol residing in both the IDL (elution time approximately 23 min) and HDL (elution time approximately 33 min) fractions. In the triglyceride percentage distributions, the reduction in VLDL was almost completely accounted for by an increase in the IDL fraction. The percentage distribution of either cholesterol or triglycerides in the LDL fraction (elution time approximately 28 min) was virtually unaffected by PD 123244-15.

Experiment 2. Although PD 123244-15 did not significantly affect egg weight, egg production was inversely
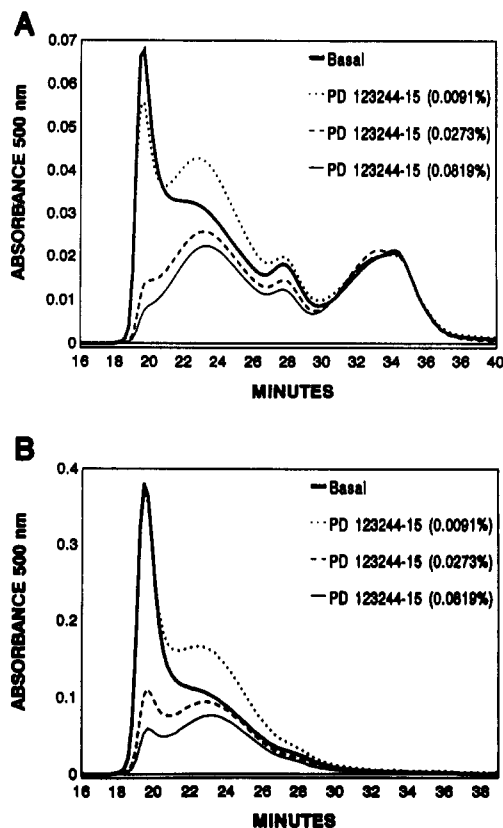

Figure 3. Plasma lipoprotein cholesterol (A) and triglyceride (B) distribution profiles of hens fed various amounts of PD 123244-15 (experiment 1). Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 21. Each profile is the mean response of five, five, five, or four samples from hens fed 0, 0.0091, 0.0273, or 0.0819% PD 123244-15, respectively, from day 1 to day 20. The precise percentage distributions for these profiles are provided in Table II.

related to the dietary level of the drug (Table III). Yolk weight was significantly depressed, and body weight gain was greatest, in hens fed 0.0819% PD 123244-15 vs the other three groups (Table III). The latter condition, which was also noted in the first experiment (Table I), probably resulted from a slightly higher accretion of body energy

Table III. Effect of PD 123244-15 on Laying Hen Performance (Experiment 2)[a]

diet	PD 123244-15, %	hen–day egg production, %	egg wt, g	yolk wt, g	body wt gain, g	daily feed consumption, g/hen
1	0	88.9 ± 1.3[a]	60.7 ± 0.2[a]	16.9 ± 0.2[a]	99.7 ± 10.7[ab]	108.4 ± 2.4[a]
2	0.0273	86.5 ± 1.4[ab]	59.5 ± 0.2[a]	16.7 ± 0.1[a]	78.5 ± 9.8[b]	96.6 ± 1.5[b]
3	0.0546	80.6 ± 1.5[bc]	58.6 ± 0.2[a]	16.5 ± 0.1[a]	105.3 ± 22.8[ab]	101.6 ± 2.2[b]
4	0.0819	75.0 ± 2.3[c]	58.0 ± 0.3[a]	15.7 ± 0.2[b]	138.7 ± 25.9[a]	101.9 ± 2.2[b]

[a] The duration of the experiment was 42 days. Data are presented as means ± SE. Hen–day egg production, body weight gain, and feed consumption data are the mean values of six hens per diet. Egg weights are the mean values of 224, 218, 203, or 189 eggs from hens fed diets 1, 2, 3, or 4, respectively. Yolk weights are the mean values of 36 yolks per diet. Means within each column with different superscripts are significantly different ($P \leq 0.05$).
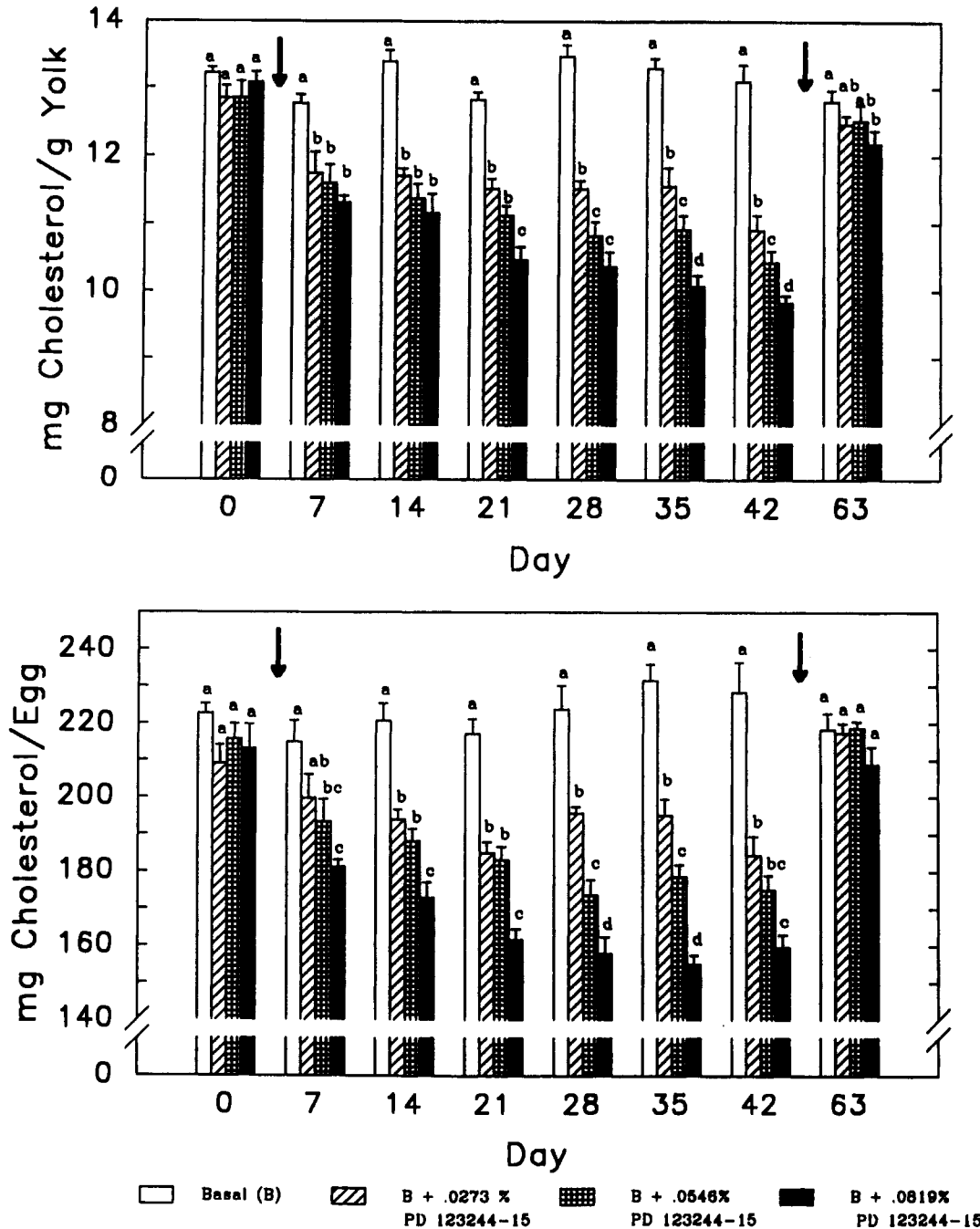

Figure 4. Mean (±SE) cholesterol content, expressed on a per gram of yolk or per egg basis, of eggs from hens fed various amounts of PD 123244-15 (experiment 2). Arrows indicate dietary inclusion (day 1) and withdrawal (day 43) of the drug. Each bar is the mean value of six eggs each. Within a day, bars with different letters are significantly different ($P < 0.05$).

and protein reserves due to the lesser demand for nutrient deposition into eggs.

Egg cholesterol contents varied inversely with the dietary level of PD 123244-15 (Figure 4). A drug effect was noted as early as day 7, and maximum reductions in cholesterol content (vs day 0 values) of approximately 25% (day 42)

and 27% (day 35) on a per gram of yolk and a per egg basis, respectively, were observed in birds fed 0.0819% PD 123244-15.

Plasma total cholesterol and triglyceride concentrations varied inversely with the dietary drug level, with hens fed 0.0819% PD 123244-15 exhibiting approximately 46 and
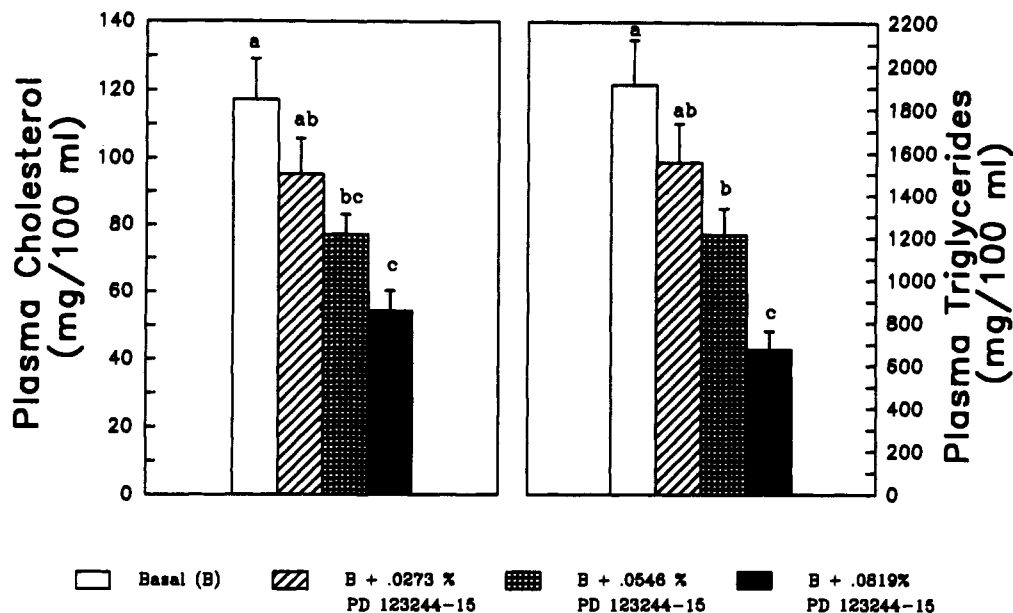
Figure 5. Mean (±SE) plasma total cholesterol and triglyceride concentrations of hens fed various amounts of PD 123244-15 (experiment 2). Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 43. Each bar is the mean value of six samples each from hens fed their respective diets from day 1 to day 42. Bars with different letters are significantly different (*P* < 0.05).

35% of the control values, respectively (Figure 5). Plasma analyses by HPGC confirmed the results of the first experiment in that the PD 123244-15-mediated lowering of plasma total cholesterol and triglyceride concentrations was almost completely accounted for by a reduction in the VLDL fraction (elution time approximately 20 min; Figure 6). On a percentage distribution basis (Table IV), this resulted in an increase in the amount of cholesterol residing in both the IDL (elution time approximately 23 min) and HDL (elution time approximately 34 min) fractions with increasing dietary levels of PD 123244-15. In the triglyceride percentage distributions, the reduction in VLDL was almost completely accounted for by an increase in the IDL fraction. PD 123244-15 caused slight, but significant, increases in the cholesterol and triglyceride LDL fractions (elution time approximately 28 min).

A representative standard chromatogram, as well as those of yolk and albumen extracts of eggs from hens fed 0 or 0.0819% PD 123244-15, is shown in Figure 7. PD 123244-15 apparently was not transferred to either egg component. However, since the detection limit of this procedure was approximately 15 ng/injection and recoveries of PD 123244-15 added to yolk or albumen samples averaged 51 and 57%, respectively (possibly due to lactonization of the open chain analog of the β-hydroxy-δ-lactone portion of the molecule during the extraction step), it was concluded that if PD 123244-15 or any UV-absorbing metabolites were transferred to the egg yolk or albumen, they were present at concentrations of less than approximately 6 ppm.

DISCUSSION

In laying hens, cholesterol is primarily biosynthesized in the liver (Andrews et al., 1968) and secreted into the plasma in the form of VLDL (George et al., 1987; Burley et al., 1984). VLDL are then transported to the ovary, where they are bound and taken up by growing oocytes via receptor-mediated endocytosis (Nimpf and Schneider, 1991). Thus, the liver is the main source of most lipid found in egg yolk (Hargis, 1988; Noble et al., 1990).

The results of the present study suggest that PD 123244-15 reduces egg yolk cholesterol content by impairing hepatic cholesterol and/or VLDL synthesis. This hy-
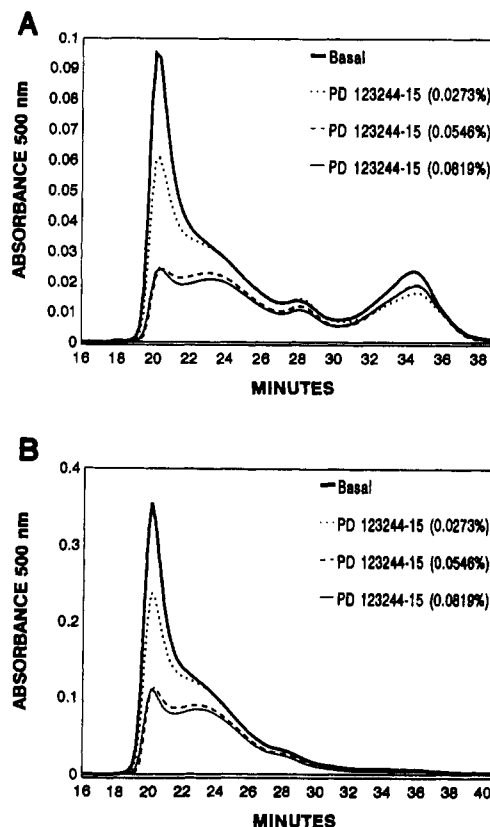

Figure 6. Plasma lipoprotein cholesterol (A) and triglyceride (B) distribution profiles of hens fed various amounts of PD 123244-15 (experiment 2). Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 43. Each profile is the mean response of six samples each from hens fed their respective diets from day 1 to day 42. The precise percentage distributions for these profiles are provided in Table IV.

pothesis is in keeping with the concept that cholesterol is an obligatory component for normal assembly of VLDL and that inhibition of cholesterol biosynthesis limits the availability of cholesterol for production and secretion of VLDL (Khan et al., 1990; Kasim et al., 1992b). Furthermore, Arad et al. (1992) recently reported that the overall hypolipidemic effects of lovastatin in patients with com-

Table IV. Percentage Distributions of Plasma Lipoprotein Cholesterol and Triglycerides in Laying Hens Fed Various Amounts of PD 123244-15 (Experiment 2)[a]

diet	PD 123244-15, %	cholesterol				triglycerides			
		VLDL, %	IDL, %	LDL, %	HDL, %	VLDL, %	IDL, %	LDL, %	HDL, %
1	0	38.4 ± 2.8[a]	27.3 ± 2.3[a]	8.4 ± 0.4[c]	25.9 ± 4.3[a]	52.0 ± 2.5[a]	36.2 ± 2.0[b]	8.4 ± 0.5[b]	3.4 ± 0.3[a]
2	0.0273	28.0 ± 3.4[b]	32.8 ± 3.5[a]	11.9 ± 0.8[ab]	27.3 ± 5.2[a]	43.4 ± 3.3[a]	43.7 ± 3.2[b]	10.8 ± 0.3[ab]	2.1 ± 0.3[a]
3	0.0546	15.0 ± 2.9[c]	36.0 ± 4.7[a]	13.6 ± 1.1[a]	35.4 ± 5.2[a]	27.6 ± 3.2[b]	57.7 ± 3.8[a]	12.0 ± 1.4[a]	2.7 ± 0.5[a]
4	0.0819	14.9 ± 3.8[c]	36.6 ± 3.4[a]	11.1 ● 0.5[b]	37.4 ● 5.4[a]	24.2 ± 4.7[b]	60.9 ± 4.1[a]	12.2 ± 0.9[a]	2.7 ± 0.8[a]

[a] Data are from the profiles depicted in Figure 6. Hens were fasted for 15 h prior to procurement of the blood samples at 8:00 a.m. on day 43. Each value is the mean (±SE) of six samples each from hens fed their respective diets from day 1 to day 42. Within a column, means with different superscripts are significantly different ($P \leq 0.05$).
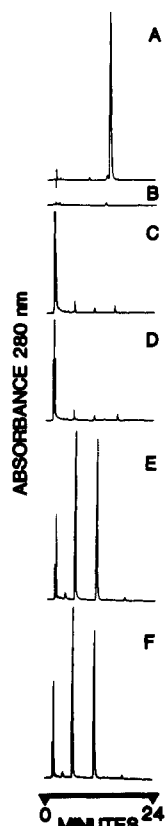

Figure 7. Reversed-phase HPLC elution profiles (experiment 2): (A) PD 123244-15 standard injection (1 μg/10-μL injection volume); (B) mobile phase (blank) injection; (C) egg albumen extract from a hen fed the basal diet; (D) egg albumen extract from a hen fed the basal diet supplemented with 0.0819% PD 123244-15; (E) egg yolk extract from a hen fed the basal diet; (F) egg yolk extract from a hen fed the basal diet supplemented with 0.0819% PD 123244-15. The column used was a 4.6 × 250 mm Beckman Ultrasphere ODS (5 μm). The mobile phase (flow rate, 1.2 mL/min) consisted of acetonitrile–water–acetic acid (50:49.85: 0.15 v/v/v). The detection limit of the assay was approximately 15 ng/10-μL injection.

bined hyperlipidemia were due to decreased assembly and secretion of apo-B-containing lipoproteins. They also observed no consistent changes in the fractional catabolic rates of either VLDL triglyceride or VLDL apo-B during lovastatin therapy.

Although the various peaks in the plasma cholesterol and triglyceride distribution profiles are designated VLDL, IDL, LDL, or HDL, the HPGC technique employed in the present study separates lipoprotein fractions primarily on the basis of size (Kieft et al., 1991). Since exhaustive compositional analyses of the various lipoprotein fractions were not conducted, it may be alternately concluded that PD 123244-15 caused a reduction in both the amount and size of the VLDL particles produced. Confirmatory evidence for an effect of HMG-CoA reductase inhibitors on VLDL particle size was recently reported in Zucker-obese rats by Kasim et al. (1992a), who concluded that

lovastatin interfered with the hepatic secretion of VLDL triglyceride, but not VLDL apo-B, resulting in the production of smaller, triglyceride-depleted VLDL particles.

The present paper, which describes the largest and most sustained reduction of egg yolk cholesterol content achieved to date, suggests that HMG-CoA reductase inhibitors hold promise in this regard. In addition, since the limit to which egg cholesterol levels can be lowered is unknown, it is possible that reductions even greater than those observed with PD 123244-15 may be attained, considering that new hypocholesterolemic drugs are constantly being developed, evaluated, and introduced by the pharmaceutical industry. However, since cholesterol is needed by the hen for the synthesis of steroid hormones necessary for the maintenance of egg production, any substance that might be useful in reducing cholesterol biosynthesis and, therefore, limiting egg cholesterol content, would need to be employed at levels that would permit sufficient cholesterol synthesis to meet the physiological needs for this substance (Naber, 1976).

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