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Received July 26, 1956. P.S.E.B.M., 1956, v93.

Anti-Inflammatory Protection of Guinea Pigs by Egg Yolk: Effect on Adrenals and Serum Constituents. (22758)

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Coburn *et al.*(3) have shown that dietary egg yolk reduces the intensity of the inflammatory response induced by passive anaphylactic arthritis in young guinea pigs. Their observations indicated that the active material of egg yolk resides in the alcohol soluble (crude phosphatide) fraction and is absent from both the acetone solubles (neutral fats and cholesterol) and the yolk protein. We have confirmed the above findings(19) using the anti-inflammatory bio-assay method of French *et al.*(8). The adrenal hormone, cortisone, and its related steroids have been used in treatment of inflammatory diseases(15,18). Their protection of laboratory animals against inflammation induced by BCG(21), tuberculin(2), turpentine(16), and Pneumococci(22) has also been demonstrated. French *et al.* (8) showed that cortisone treatment also reduced swelling in joints of guinea pigs which had been subjected to a reverse Arthus reaction.

The present investigation was undertaken (a) to compare the anti-inflammatory protection by cortisone to that afforded by dietary egg yolk, (b) to study the effect of yolk alcohol solubles on adrenals of the guinea pig, and (c) the effect of egg yolk on blood constituents of guinea pigs.

Methods. 1. *Fractionation of egg yolk powder.*† Acetone solubles, alcohol solubles and the protein fraction of egg yolk were obtained by procedures of Coburn *et al.*(3). 2. *Assaying procedure.* Weanling guinea pigs weighing about 160 g (unless otherwise indicated) were used as test animals in groups of 6 or more. Each animal was weighed just prior to, and every 3 days during the experiment, and daily food intake was recorded. Egg yolk and yolk fractions were individually fed to guinea pigs as supplements to a basal control diet for a period of 21 to 26 days. The basal diet contained 50% Rockland guinea pig diet and 50% dixie rabbit pellets (22% protein) to which was added 0.1% ascorbic acid. At end of the feeding period, the guinea pigs usually reached the desired weight of approximately 300 g and then used for bio-assay(8). For each assay group, a control group of 6 or more guinea pigs was fed and challenged at the same time to minimize environmental influences on inflammation.

Results. Exp. 1 was a comparison of the anti-inflammatory activity of egg yolk and yolk alcohol solubles to that of cortisone. Cortisone acetate was injected intraperitoneally into 300 g guinea pigs (after 3-4 days on basal diet) 1 hr prior to assay. For each assay a second group of guinea pigs, pretreated with saline, was used as control. Feeding dried egg yolk at 19.5%, or yolk alcohol solubles at 2-8% of diet, resulted in 28-40% inhibition of swelling (Table I) similar to that obtained by administering cortisone acetate at the level of 25 mg/kg body weight(8). When alcohol solubles were fed at less than 2% of diet, no protection was observed.

Exp. 2 was a study of the effect of dietary yolk alcohol solubles on adrenals of the guinea

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TABLE I. Effect of Feeding Dried Egg Yolk Powder and Yolk Alcohol Solubles on Mean Swelling Index (M.S.I.) in Reverse Arthus Reaction of Guinea Pigs.

No. guinea pigs	Fed control, M.S.I. ± S.E.	No. guinea pigs	Yolk powder, 19.5%	Yolk alcohol solubles 1%	2%	4%	6%	8%	% inhibition
				M.S.I. ± S.E.					
6	4.8 ± .37	8	3.4 ± .37						28
7	4.8 ± .34	6		5.6 ± .43					0
6	6.0 ± .30	5			4.3 ± .59				28
6	4.0 ± .37	7				3.0 ± .53			25
6	6.0 ± .30	6					3.9 ± .77		35
7	4.6 ± .41	6						3.4 ± .18	26
6	4.8 ± .31	6						2.9 ± .20	40
6	5.2 ± .50	7						3.1 ± .50	40

pig. Guinea pigs weighing 160 or 200 g were fed for periods of 21 and 10 days respectively. At completion of the assay, the guinea pigs were killed by blow on head. The adrenals were quickly removed, cleaned from adherent tissues, washed with physiological saline, weighed and frozen. They were kept in frozen state until needed. The adrenals in each group were pooled and water content was determined(1). The total lipid content was determined on ground, dried adrenals by extraction with hot chloroform and methanol (2:1) in Soxhlet extractor for 36 hrs. The extract was evaporated to dryness in water bath at 40°C under stream of nitrogen. The residue was redissolved in chloroform at room temperature and filtered. The total lipid was calculated from the weight of residue remaining after filtrate was dried to a constant weight. The total cholesterol in lipid extract was then determined(23).

The similarity between the anti-inflammatory protection afforded the guinea pig by dietary egg yolk and by injected cortisone acetate suggested that the egg yolk effect might be mediated by the adrenals. However, no indication of increased adrenal hormone production was observed in the treated guinea pigs (Table II). Adrenal weights were not increased nor were adrenal cholesterol levels depressed by feeding yolk alcohol solubles.

Exp. 3. The effect of dietary egg yolk on certain blood constituents of guinea pigs was studied. Serum lipids of guinea pigs, fed egg yolk, or 1 of 3 egg yolk fractions (alcohol solubles, acetone solubles and protein fraction) were determined. Each experimental diet contained one or another of the 3 fractions in a quantity equivalent to that which would be found if 19.5% of diet weight were egg yolk. In studying the effect of egg yolk on serum total N and non-protein N levels, the guinea pigs were fed the alcohol soluble fraction. Heart blood samples were obtained from fasting animals 24 hrs after completion of the assay. Total lipid was determined by extracting lyophilized serum with hot chloroform and methanol (2:1) as described for determination of adrenal lipid content. Lipid phosphorus and serum cholesterol were then determined(11,23). Phospholipid values were calculated by multiplying lipid phosphorus by 26. The serum total N and non-protein N of the protein-free filtrate(7) were determined by micro-Kjeldahl(13,14). No essential dif-

TABLE II. Effect of Yolk Alcohol Solubles on Adrenals of 6 Guinea Pigs in Each Series.

Treatment	Days	Wt, mg/100 g body wt	Water, %	Lipid, %*	Cholesterol, %†
Control	21	46.5 ± 4.5	57.1	50.5	25.3
Yolk alcohol solubles, 8%		38.3 ± 2.8	56.6	48.2	25.6
Control	10	37.1 ± 4.1	65.6	54.6	17.2
Yolk alcohol solubles, 2%		42.5 ± 3.5	63.7	47.2	20.7
" " " , 6%		42.3 ± 2.8	63.6	49.6	20.1

* Expressed as % of dry wt of adrenals.
† " " " " " lipid content of adrenals.

TABLE III. Serum Total Lipid, Phospholipid and Cholesterol Levels of Guinea Pigs on Control and Supplemented Diets.

Treatment	Total lipid, mg/ml	P	Phospholipid, mg/ml	P	Total cholesterol, mg/ml	P	C/P
Control	2.03 ± .12*		.384 ± .04*		.466 ± .07*		1.21
Dried yolk powder	4.79 ± .92	.0125	.483 ± .08	>.1	.606 ± .08	>.1	1.25
Control	2.14 ± .22		.324 ± .06		.258 ± .07		.80
Yolk protein	14.60 ±1.17	.0025	3.774 ± .42	<.0025	7.410 ± .34	<.0025	1.96
Control	2.07 ± .22		.376 ± .04		.437 ± .05		1.16
Yolk alcohol solubles	3.55 ± .71	.025	.533 ± .05	.05	.468 ± .03	>.1	0.91
Control	2.78 ± .09		.406 ± .04		.354 ± .02		0.87
Yolk acetone solubles	4.42 ± .80	.025	1.074 ± .29	.025	1.046 ± .31	<.025	0.97

* ± S.E.

ference was found in average daily food intake and weight gain between guinea pigs fed control or supplemented diets. Animals, which were fed yolk alcohol solubles, received the same protection as those fed a supplement of dried yolk powder. Both the protein and the acetone soluble fractions appeared inactive.

Guinea pigs which had received acetone soluble material possessed enlarged fatty livers and gallbladders. Guinea pigs from other groups had normal organs.

Feeding dried egg yolk and yolk alcohol-solubles resulted in elevation of serum total lipid with no increase in cholesterol to phospholipid ratio (Table III). Feeding inactive yolk fractions caused elevation of serum total lipids, phospholipids, cholesterol, and the cholesterol to phospholipid ratio. The serum total N and non-protein N were unaffected by feeding yolk alcohol solubles.

Discussion. An anti-inflammatory effect of phospholipid, sphingomyelin has been demonstrated by several workers. Kerr *et al.*(12) showed that sphingosine, as well as sphingomyelin, inhibited the skin reaction in cattle sensitized to *Trichomonas foetus* antigen. The favorable effect of sphingomyelin and sphingosine has also been observed in guinea pigs sensitized to tuberculin(6).

In preliminary fractionation of alcohol solubles of egg yolk, we found that the active factor was present in the remaining fraction of alcohol solubles, after lecithin had been precipitated. The lecithin fraction which was obtained by a cadmium chloride precipitation did not appear to be active. The possibility that the active yolk material is also associated with the crude sphingomyelin fraction has been suggested by Coburn *et al.*(3).

Of the serum constituents studied, the total lipid fraction was elevated by feeding egg yolk or yolk alcohol solubles. Serum phospholipids and cholesterol were slightly elevated by these two diet supplements. Therefore, it seems that elevation of certain serum lipids other than cholesterol and phospholipids accounts for the hyperlipemia observed after feeding active egg yolk fractions. Hyperlipemia produced by active yolk material is quite comparable to that observed in rat, rabbit and human after administration of cortisone(5,4,10,20). These reports indicated that administration of cortisone caused hyperlipemia due chiefly to increase of lipids other than cholesterol and phospholipids. The inactive yolk fractions both caused an increase in serum total lipids proportional to the increase of phospholipids and cholesterol. If the inhibitory effect of the active factor on the experimental inflammation in guinea pigs depends on changes in the blood lipid fractions, then it seems logical to attribute its effect to the associated increase in lipid fractions other than cholesterol and phospholipids. However, the possibility that active yolk material inhibits induced inflammation independent of its effect on blood lipids cannot be excluded at present.

Summary. The anti-inflammatory activity of egg yolk has been studied in weanling

guinea pigs. 1. The active material resided in the alcohol soluble fraction and was absent from the protein and acetone soluble fractions. 2. The anti-inflammatory effect did not appear to be mediated by the adrenals. 3. Feeding egg yolk and yolk alcohol solubles resulted in an elevation of serum total lipids with no increase in cholesterol to phospholipid ratio. Inactive yolk fractions caused elevations of serum total lipids, phospholipids, cholesterol, and cholesterol to phospholipid ratio. 4. Serum levels of total N and non-protein N were unaffected by yolk alcohol solubles.

The authors wish to express their thanks to Drs. John R. Allen and Joseph C. Street for their assistance in preparing egg yolk fractions and conducting the anti-inflammatory assays.

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Received August 8, 1956. P.S.E.B.M., 1956, v93.

Δ^1-Hydrocortisone and Hydrocortisone: Plasma 17-Hydroxycorticosteroid Concentrations in the Dog Following Oral and Intravenous Administration. (22759)

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Δ^1-Hydrocortisone ($\Delta^{1,4}$-pregnadiene-11β, 17α, 21-triol-3,20-dione) has received considerable attention as a potent synthetic analogue of hydrocortisone (Δ^4-pregnene-11β, 17α, 21-triol-3, 20-dione). Clinical studies have indicated Δ^1-hydrocortisone to be 3 to 5 times as potent as the parent steroid(1-3). Evaluation of glucocorticoid activity (liver glycogen deposition, eosinopenic response, thymus involution) has shown a similar ratio (4). Introduction of unsaturation at the C_1 position of the steroid A-ring could conceivably alter the rates of absorption and/or metabolism of the compound. In the present study, this possibility has been examined experimentally in the dog by determining the changes in plasma 17-hydroxycorticosteroid (17-OHCS) concentrations as a function of time following oral and intravenous administration of Δ^1-hydrocortisone and hydrocortisone. Subsequent to this study, a similar evaluation of Δ^1-hydrocortisone in the human has been reported(5).

Materials and methods. Male mongrel