IMMUNOLOGY

Vitamin E as Adjuvant in Emulsified Vaccine for Chicks[1]

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ABSTRACT Mineral oil was partially replaced with D, L-α-tocopheryl acetate (vitamin E) in bacterial and viral inactivated emulsified vaccines. Vitamin E increased the immune response to the viral antigen (Newcastle disease virus) used but not to the bacterial antigen (*Escherichia coli*) when its presence in the oil phase did not exceed 30%. Inoculated vitamin E may have enhanced the immune response by interacting with the immune-competent cells involved in the inflammatory reaction that followed inoculation of emulsified vaccines.

(*Key words*: vitamin E, emulsified vaccines, mineral oil, immune response, chicks)

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INTRODUCTION

Many studies on the biological activities of vitamin E have been carried out since it was first discovered by Evans and Bishop (1923). More recently, researchers have devoted special attention to the influence of vitamin E on the immune system. High doses of vitamin E increased antibody production stimulated by various antigens (Tengerdy *et al.*, 1973, 1981; Tengerdy and Brown, 1977), increased resistance to bacterial infections, and reduced mortality rates (Heinzerling *et al.*, 1974a,b; Tengerdy and Nockels, 1975; Tengerdy and Brown, 1977).

Vitamin E is particularly important in the development of the immune system of the chicken (Marsh *et al.*, 1981; Franchini *et al.*, 1986), as it enhances lymphocyte proliferation (Tanaka *et al.*, 1979; Corwin and Shloss, 1980; Corwin and Gordon, 1982). Vitamin E also stimulates the proliferation of T-lymphocytes, particularly helper T-cells (Tanaka *et al.*, 1979), annuls the effect of particular substances such as spermine products, which inhibit *in vitro* mitogenesis of T-lymphocytes (Corwin and Gordon, 1982), and positively influences cooperation between T- and B-lymphocytes (Tanaka *et al.*, 1979). Vitamin E also favors the proliferation of macrophages, increasing their functional capacities and enhancing the activity of Ia surface antigens responsible for cooperation between immune-competent cells, resulting in antibody response (Gebremichael *et al.*, 1984).

One of the main effects of vitamin E on the immune system is due to inhibition of prostaglandin biosynthesis. This occurs by means of a mechanism acting on the metabolism of polyunsaturated fatty acids, particularly arachidonic acid. Arachidonic acid is known to be converted by the enzymes lipoxygenase and cycloxygenase into hydroperoxides and endoperoxides, which in turn produce prostacyclines, tromboxanes, and prostaglandins (Panganamala and Cornwell, 1982). The inhibiting effect of vitamin E on prostaglandin biosynthesis is followed by increased humoral and cell-mediated immunity and phagocytosis. The E prostaglandins (PGE) interfere with various immunological functions, such as the cytotoxic activity of lymphocytes, mitogen-mediated proliferation of lymphocytes (Goodwin *et al.*, 1977; Goodwin and Webb, 1980), and the various functions carried out by macrophages in the complex mechanism of the immune response (Snyder *et al.*, 1982, Metzger *et al.*, 1982; Gebremichael *et al.*, 1984). Prostaglandins also act as endogenic modulators on the

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immune response. For example, PGE_1, produced in small quantities by leucocytes in induced mitogenesis, inhibits the effect of the phytohemagglutinin (Goodwin *et al.*, 1977). Proliferating macrophage populations also contain cells called "suppressors"; their production of PGE_2 inhibits further proliferation of macrophages and their immune functions (Goodwin and Webb, 1980; Metzger *et al.*, 1982).

The aim of the present research was to verify the effects on the immune response when light mineral oils in emulsified vaccines, prepared with inactivated bacteria and viruses, were partially replaced by vitamin E. In birds, emulsified vaccines mainly act by means of two of the three mechanisms indicated by Freund (1956): one linked to prolonged elution of the antigen, due to the slow resolution of the emulsion, the other to the accumulation of the immune-competent cells (macrophages and lymphocytes) around the emulsion at the point of inoculation, due to an inflammatory granulomatous reaction.

TABLE 1. *Composition of feed*

Ingredients and calculated composition	0 to 29 days	29 to 105 days
	(%)	
Corn meal	58.38	63.38
Soybean meal (48% CP)	33.33	25.82
Fat	2.56	3.09
Corn gluten	1.83	4.06
Dicalcium phosphate	2.01	1.90
Calcium carbonate	1.05	.95
Sodium chloride	.35	.35
DL-methionine	.17	.13
Choline chloride	.15	.15
Vitamin premix[1]	.033	.033
Mineral premix[2]	.112	.112
Ethoxyquin	.025	.025
Calculated composition		
CP	22.93	19.99
ME, kcal/kg	3,101	3,201
Calorie:protein ratio	141	160
Fat	5.06	5.72
Calcium	.97	.90
Total phosphorus	.73	.69
Available phosphorus	.46	.44
Lysine	1.18	.98
Methionine	.50	.54
Methionine plus cystine	.86	.80

[1]Vitamin premix supplied per kilogram of diet: vitamin A, 11,000 IU; cholecalciferol, 3,000 IU; vitamin E, 15 mg; vitamin K_3, 2 mg; vitamin B_1, 3 mg; vitamin B_2, 8 mg; pantothenic acid, 17 mg; nicotinic acid, 60 mg; vitamin B_6, 6 mg; vitamin B_{12}, .02 mg; folic acid, 1 mg; biotin, .2 mg; and vitamin C, 25 mg.

[2]Mineral premix supplied per kilogram of diet: Se, .10 mg; Zn, 55 mg; Mn, 55 mg; Fe, 60 mg; Cu, 2.8 mg; Co, .1 mg; I, .5 mg.

MATERIALS AND METHODS

Four hundred and twenty one-day-old Arbor Acres chicks (210 males and 210 females) were divided into six groups of 70 chicks each, in an experiment lasting 105 days. Chicks were fed for *ad libitum* consumption with a practical diet as shown in Table 1.

At 21 days of age, all the chicks, housed in cages, were inoculated (.5 mL per bird) subcutaneously in the retronucal area with six different emulsified vaccines, each containing Newcastle disease virus (NDV) and a strain of *Escherichia coli* inactivated with betapropiolactone. The vaccines differed in the characteristics of their oily phase: the vaccine given to Group 6 (the control group) contained only light mineral oils, whereas in Groups 1 to 5 the mineral oil was replaced by various percentages of vitamin E in the form of D,L-α-tocopheryl-acetate (Table 2). One sample of each vaccine was used to determine the content of vitamin E by high pressure liquid chromatography (Cohen and Lapointe, 1980).

One blood sample was taken from each chick at the moment of vaccination and on Days 7, 14, 21, 28, 38, 48, 58, 68 and 84 postinoculation (PI). The sera were individually titered for antibodies inhibiting hemoagglutination inhibition (HI) of NDV by the micromethod, using Procedure β and 10 Ha units (Anonymous, 1971), and for the *Escherichia coli* agglutinating antibodies by microagglutination. The HI and agglutinin titers were

TABLE 2. *Amount of vitamin E (D,L-α-tocopheril acetate) added to vaccines and the amount of vitamin E in each vaccinal dose*

Vaccine	Oily components		Vitamin E per dose
	Mineral oil	Vitamin E	
	(%)		(mg)
1	90	10	36
2	80	20	74
3	70	30	116
4	60	40	150
5	50	50	188
6	100	0	0

transformed into the logarithm of reciprocal dilution (White, 1973).

Ten, 20, and 30 days after vaccination, 10 chicks (5 males and 5 females) from each of the six inoculated groups were killed. Post-mortem examinations were carried out to search for local or general lesions caused by inoculation. Samples of neck tissue were fixed in formalin, embedded in paraffin, sectioned, and then stained with hematoxylin-eosin and Mallory's dye.

Statistical Analysis

The titers were analyzed the General Linear Models procedure (SAS Institute, 1985) with the following model:

$$Y_{ijk} = \mu + D_i + S_j + DS_{ij} + e_{ijk}$$

where: Y_{ijk} = value of the 1st chick titer; μ = population mean; D_i = effect of the ith dose (i = 0, 10, 20, 30, 40, and 50% of vitamin E); S_j = effect of the jth sex (j = male, female); DS_{ij} = two-way interaction effect of the ith dose and jth sex; and e_{ijk} = residual error. Differences between means were tested with the Student-Newman-Keuls Test.

RESULTS AND DISCUSSION

The production of HI antibodies to NDV was higher ($P<.01$) in the chicks of Groups 2, 3, and 1 (inoculated with vaccines in which the mineral oil had been replaced, respectively, by 20, 30, and 10% vitamin E) than in those of control Group 6, which had received a vaccine whose oily phase was composed of light mineral oils only (Table 3). Birds in Group 4, inoculated with a vaccine with 40% vitamin E had HI antibodies similar to the control Group 6 except on Day 28 PI. The HI titers in the birds of Group 5, inoculated with a vaccine with 50% vitamin E, indicated that this group did not produce any specific circulating antibodies. The HI titers in Groups 2 and 3 (20 and 30% vitamin E) were generally higher than those in Group 1 (10% vitamin E), with significant difference occurring only at Days 21 and 38 PI. Although HI titers were generally higher in Group 2 than in Group 3, differences were not statistically significant.

The influence of sex on response to the NDV vaccine seemed to be correlated with days PI. Higher HI titers were observed in females than in males during the later samplings (Days 58 and 84 PI) (Table 3). Statistically higher antibody titers in females were observed in other studies (Franchini *et al.*, 1988, 1989). Probably the sex difference in titers were related to similar sex difference in plasma protein levels (Sturkie and Newman, 1951), due to the fact that sex hormones, especially estrogens (Sturkie and Newman, 1951), favor higher serum globulin levels in females.

The immune response to *E. coli* did not show any beneficial effect due to replacement of part of the mineral oil with vitamin E (Table 4). As observed in a previous experiment (Franchini *et al.*, 1988), in which a strain of *Pasteurella anatipestifer* Serotype 1 was used as bacterial antigen, the agglutinating titers of the animals in control Group 6, inoculated with a vaccine containing only mineral oils, were generally higher and on some occasions (Days 14, 21, 28, and 38 PI) the differences were statistically significant.

The post-mortem findings on the animals killed 10, 20, and 30 days PI showed severe granulomatous lesions in Groups 1, 2, and 3, inoculated with vaccines containing 10, 20, and 30% vitamin E, respectively, and in control Group 6. In Groups 4 and 5 (40 and 50% vitamin E, respectively) the lesions were smaller and tended to heal with time, until they had almost disappeared on 30 days PI.

Histological findings substantially confirmed macroscopic observations. Inflammation was more marked in Groups 1, 2, 3, and 6 (control). All these chicks, killed 10 and 20 days PI, systematically showed granulomas composed of a necrotic core, with giant epithelial cells, surrounded by young granulating tissue that, in some cases, was so abundant that it enclosed large structures such as blood vessels, nerves, cutaneous muscles, and the thymus. Thirty days PI, granulomas of this type were found together with optically empty structures like cysts, probably containing drops of adjuvant, generally immersed in connective tissue, mainly infiltrated by monocytes and rich in vacuoles. In Groups 4 and 5 (40 and 50% vitamin E), the inflammatory reaction was found to be less intense even at the first sampling 10 days PI. It decreased progressively until it could no longer be seen in chicks killed at Day 30 PI.

The present study attempted to verify whether vitamin E, inoculated with an emulsi-

TABLE 3. *Hemagglutination inhibition ($\bar{x} \pm SD$)[1] antibodies against Newcastle disease virus*

Group	Sex[2]	Days postinoculation									
		0	7	14	21	28	38	48	58	68	84
1	M	2.13 ± .35	2.33 ± .48	4.40 ± 1.63	5.46 ± 1.59	6.60 ± 1.05	5.14 ± 1.09	3.14 ± 1.29	4.07 ± .95	4.21 ± .89	3.85 ± 1.12
(10% vitamin E)	F	2.06 ± .25	2.06 ± .25	5.33 ± 1.39	5.86 ± 1.45	7.20 ± 1.01	5.86 ± 1.12	4.06 ± .88	5.53 ± .91	5.57 ± 1.34	5.13 ± 1.12
	$\bar{x}$	2.10 ± .30	2.20 ± .41^{A}	4.86 ± 1.56^{AB}	5.66 ± 1.51^{B}	6.90 ± 1.06^{A}	5.51 ± 1.15^{BC}	3.10 ± 1.08^{A}	4.85 ± 1.17^{A}	4.89 ± 1.31^{AB}	4.72 ± 1.27^{A}
2	M	2.13 ± .35	2.00 ± .00	6.66 ± 1.87	7.53 ± 1.88	8.00 ± 1.10	7.07 ± 1.03	5.46 ± 1.12	4.92 ± .64	5.00 ± 1.09	3.50 ± 1.00
(20% vitamin E)	F	2.13 ± .35	2.06 ± .45	4.60 ± 1.84	6.06 ± 2.01	7.20 ± 1.32	5.66 ± 1.15	4.92 ± .99	5.00 ± 1.06	5.61 ± 1.12	4.92 ± 1.18
	$\bar{x}$	2.13 ± .34	2.03 ± .31^{AB}	5.63 ± 2.10^{A}	6.80 ± 2.05^{A}	7.58 ± 1.26^{A}	6.40 ± 1.29^{A}	5.20 ± 1.08^{A}	4.96 ± .88^{A}	5.33 ± 1.12^{A}	4.58 ± 1.27^{A}
3	M	2.06 ± .25	2.06 ± .25	5.13 ± 1.72	6.46 ± 1.88	6.93 ± 1.16	5.66 ± .97	4.53 ± .83	4.28 ± 1.13	4.73 ± 1.03	3.66 ± 1.22
(30% vitamin E)	F	2.13 ± .35	2.00 ± .37	5.80 ± 1.82	7.20 ± 1.47	7.06 ± 1.03	5.80 ± .77	5.20 ± .56	5.00 ± .84	5.60 ± .73	4.85 ± .66
	$\bar{x}$	2.10 ± .30	2.03 ± .31^{AB}	5.46 ± 1.77^{A}	6.83 ± 1.70^{A}	7.00 ± 1.08^{A}	5.73 ± .86^{AB}	4.86 ± .77^{A}	4.65 ± 1.04^{A}	5.16 ± .98^{A}	4.39 ± 1.07^{A}
4	M	2.20 ± .41	1.93 ± .25	3.06 ± 1.43	4.66 ± 1.11	6.33 ± .72	5.20 ± .86	4.13 ± .83	3.71 ± .82	4.35 ± 1.15	3.45 ± .82
(40% vitamin E)	F	2.00 ± .00	1.93 ± .25	3.28 ± 1.72	4.28 ± 1.58	5.46 ± .91	4.46 ± .99	3.71 ± .91	3.66 ± .97	4.21 ± .97	3.40 ± .98
	$\bar{x}$	2.10 ± .30	1.93 ± .25^{B}	3.17 ± 1.55^{C}	4.48 ± 1.35^{C}	5.90 ± .92^{B}	4.83 ± .98^{C}	3.93 ± .88^{B}	3.68 ± .89^{B}	4.28 ± .99^{BC}	3.42 ± .90^{B}
5	M	2.20 ± .41	1.93 ± .25	1.26 ± .79	1.64 ± 1.00	1.64 ± 1.27	1.38 ± .76	1.66 ± .72	1.26 ± .45	1.37 ± .74	1.66 ± .81
(50% vitamin E)	F	2.13 ± .35	1.87 ± .35	1.00 ± .00	1.21 ± .42	1.00 ± .00	1.00 ± .00	1.46 ± .87	1.76 ± .72	1.30 ± .75	1.28 ± .61
	$\bar{x}$	2.16 ± .37	1.90 ± .30^{B}	1.13 ± .58^{D}	1.42 ± .79^{D}	1.32 ± .94^{D}	1.17 ± .54^{D}	2.57 ± .79^{C}	1.50 ± .63^{C}	1.33 ± .73^{D}	1.40 ± .68^{C}
6	M	2.13 ± .35	1.93 ± .25	4.53 ± 1.92	5.42 ± 1.45	5.07 ± 1.32	4.53 ± 1.39	3.76 ± 1.30	3.42 ± 1.28	3.58 ± 1.31	2.77 ± 1.20
(control)	F	2.06 ± .25	1.87 ± .35	3.42 ± 1.65	4.86 ± 1.35	4.86 ± 1.06	4.92 ± 1.38	4.06 ± .96	3.53 ± .66	3.80 ± .77	2.85 ± 1.02
	$\bar{x}$	2.10 ± .30	1.90 ± .30^{B}	4.00 ± 1.85^{BC}	5.13 ± 1.40^{BC}	4.96 ± 1.17^{C}	4.74 ± 1.37^{C}	3.92 ± 1.11^{B}	3.48 ± 1.01^{B}	3.70 ± 1.03^{C}	2.82 ± 1.07^{B}
Probability for sex difference		NS	NS	NS	NS	NS	NS	NS	<.001	NS	<.002

$^{A-E}$Mean of sexes within columns with no common superscripts are significantly different (P<.01).

[1]Titers expressed as a logarithm of serum reciprocal dilution ($\log_2$).

[2]M = male; F = female.

TABLE 4. *Serum agglutining antibodies ($\bar{x} \pm SD$)[1] against* Escherichia coli

Group	Sex[2]	Days postinoculation									
		0	7	14	21	28	38	48	58	68	84
1	M	2.40 ± 1.67	4.66 ± 1.98	3.93 ± .88	2.33 ± .81	4.28 ± .61	4.00 ± 1.17	4.92 ± .73	5.00 ± 1.22	4.81 ± .98	4.83 ± .75
(10% vitamin E)	F	1.60 ± .54	4.20 ± 1.65	4.53 ± 1.12	3.33 ± 1.11	4.23 ± .92	4.66 ± 1.54	4.73 ± .79	4.93 ± .79	4.42 ± 1.39	5.50 ± .65
	$\bar{x}$	2.00 ± 1.24	4.43 ± 1.81	4.23 ± 1.04^{B}	2.83 ± 1.08^{B}	4.25 ± .76	4.34 ± 1.39^{ab}	4.82 ± .75^{ab}	4.96 ± .99^{ab}	4.60 ± 1.22^{ab}	5.30 ± .73
2	M	4.20 ± 2.04	5.66 ± 1.63	4.53 ± 1.55	3.53 ± 1.24	4.57 ± 1.15	5.07 ± .86	5.21 ± .89	5.23 ± .72	4.53 ± 1.59	5.28 ± .70
(20% vitamin E)	F	3.40 ± 2,50	4.33 ± 1.87	3.86 ± 1.06	2.73 ± .70	4.08 ± .90	3.15 ± 1.51	4.57 ± 1.01	4.35 ± .74	4.27 ± 1.10	5.84 ± .98
	$\bar{x}$	3.80 ± 2.20	5.00 ± 1.85	4.20 ± 1.34^{B}	3.13 ± 1.07^{B}	4.34 ± 1.05	4.11 ± 1.55^{b}	4.89 ± .99^{ab}	4.77 ± .84^{b}	4.42 ± 1.39^{b}	5.61 ± .97
3	M	4.20 ± 2.28	4.73 ± 1.70	4.20 ± .94	2.60 ± .63	3.86 ± .74	4.20 ± 1.01	4.40 ± .98	4.46 ± 1.12	4.85 ± .86	4.66 ± 2.00
(30% vitamin E)	F	2.40 ± 1.51	4.53 ± 1.92	4.73 ± 1.09	2.93 ± .79	4.40 ± .98	4.20 ± .77	4.80 ± 1.01	4.86 ± .74	4.73 ± 1.09	5.15 ± 1.90
	$\bar{x}$	3.30 ± 2.05	4.63 ± 1.79	4.46 ± 1.04^{B}	2.76 ± .72^{B}	4.13 ± .89	4.20 ± .88^{ab}	4.60 ± 1.00^{b}	4.66 ± .95^{b}	4.79 ± .97^{ab}	4.95 ± 1.91
4	M	3.80 ± 3.42	4.33 ± 1.17	3.93 ± 1.06	2.33 ± .97	4.40 ± .82	4.06 ± .96	5.26 ± .79	5.07 ± .91	5.07 ± 1.26	4.90 ± 1.59
(40% vitamin E)	F	3.00 ± 1.58	4.07 ± 1.59	3.84 ± 1.06	2.92 ± .61	4.28 ± .99	4.42 ± .49	5.38 ± 1.04	5.64 ± .92	4.28 ± 1.32	5.75 ± 1.13
	$\bar{x}$	3.40 ± 2.54	4.20 ± 1.37	3.89 ± 1.03^{B}	2.62 ± .86^{B}	4.34 ± .89	4.46 ± .88^{ab}	5.32 ± .90^{a}	5.35 ± .95^{ab}	4.67 ± 1.33^{ab}	5.36 ± 1.39
5	M	3.60 ± 2.30	3.46 ± 1.45	4.33 ± 1.04	2.80 ± .77	4.25 ± .62	3.92 ± .86	5.15 ± .37	5.53 ± .87	5.55 ± .88	5.60 ± .54
(50% vitamin E)	F	3.40 ± 2.07	4.60 ± 1.24	3.78 ± 1.62	2.90 ± 1.07	4.00 ± .81	4.00 ± 1.06	5.15 ± .98	5.57 ± 1.08	5.27 ± .64	4.46 ± 1.92
	$\bar{x}$	3.50 ± 2.06	4.03 ± 1.44	4.06 ± 1.36^{B}	2.86 ± .91^{B}	4.12 ± .72	3.96 ± .96^{b}	5.15 ± .73^{ab}	5.55 ± .97^{a}	5.40 ± .75^{a}	5.00 ± 1.68
6	M	3.40 ± 1.14	4.06 ± 1.48	6.20 ± 1.65	3.73 ± 1.16	4.92 ± 1.49	4.63 ± 1.28	5.07 ± 1.18	5.16 ± 1.40	4.75 ± 1.35	6.00 ± 1.41
(control)	F	3.80 ± 2.38	5.20 ± 1.61	6.21 ± 1.76	3.93 ± .88	5.00 ± .92	4.93 ± .70	5.13 ± .74	5.64 ± 1.15	4.93 ± 1.09	5.61 ± .72
	$\bar{x}$	3.60 ± 1.77	4.63 ± 1.62	6.20 ± 1.67^{A}	3.83 ± 1.01^{A}	4.96 ± 1.20	4.80 ± .98^{a}	5.10 ± .95^{ab}	5.42 ± 1.27^{ab}	4.85 ± 1.19^{ab}	5.81 ± 1.00

[a,b]Means of sexes within columns with no common superscripts are significantly different (P<.05).

[A–C]Means of sexes within columns with no common superscripts are significantly different (P<.01).

[1]Titers expressed as a logarithm of serum reciprocal dilution ($\log_2$).

[2]M = male; F = female.

fied vaccine, favors the immune response by increasing antibody production. The positive results, particularly related to viral antigens, confirm the working hypothesis and may be explained by the studies of many researchers (Goodwin *et al.*, 1977; Corwin and Shloss, 1980; Corwin and Gordon, 1982). These authors showed that vitamin E in tissue cultures was not only able to reduce the inhibiting action of certain splenic substances such as spermine products, but was also capable of reducing the mitogenesis-inhibiting action that PGE, produced by proliferating lymphocytes, exerted on the proliferation of the lymphocytes themselves.

Vitamin E enhances the function of Ia surface antigens and increases macrophage mitogenesis. This reduces the depressor effect that PGE_2-producing "suppressor" macrophages exert on the proliferation of the entire population of macrophages, which accumulate massively at the point of inoculation (Snyder *et al.*, 1982; Metzger *et al.*, 1982; Gebremichael *et al.*, 1984). Inoculated vitamin E, interacting with the inflammation that follows the inoculation of emulsified vaccines, enhances the functional and mitotic activity of the cells involved in the immunity reaction, macrophages, lymphocytes, and plasma cells, which accumulate massively at the point of injection of the inoculum (Aitken and Survashe, 1974; Asdrubali, 1986). Gross and histological findings showed that a poor inflammatory reaction was the main cause of the insufficient immunity response observed in Groups 4 and 5 (inoculated with vaccines containing 40 and 50% vitamin E, respectively).

The different response to the two antigens has also been shown in other research using chickens (Franchini *et al.*, 1986, 1988) and turkeys (Franchini *et al.*, 1989), with *P. anatipestifer* as the bacterial antigen. In the present study, vitamin E did not increase the production of agglutining antibodies and titers were slightly lower in experimental Groups 1 to 5. These results may reflect a weak association between the immune response to these bacterial antigens and the activity of T-lymphocytes influenced by vitamin E (Tanaka *et al.*, 1979). It is also important to stress the influence of vitamin E on macrophage proliferation and on the functional activity of Ia surface antigens which, although indispensable for triggering the complex mechanism of the production of antibodies to a viral antigen, are not equally indispensable in their response to a bacterial antigen (Russell, 1982; Powell, 1982).

With respect to the experiment of Franchini *et al.* (1988), another interesting point may be noted. A similar amount (50%) of vitamin E in the vaccine caused an increased response to the NDV, but only if associated with *P. anatipestifer*, not with *E. coli*. The explanation for these results may lie in the stimulation of inflammation, which is far less severe in vaccines prepared with *E. coli* than in those with *P. anatipestifer*.

The present results on the adjuvant effect of vitamin E differed from those of Tengerdy *et al.* (1983), who inoculated sheep with an emulsified vaccine prepared with Toxins C and D of *Clostridium perfringens*, and whose oily adjuvant consisted exclusively of vitamin E. The good immune response of sheep to the two toxins may be due both to their different sensitivity to the antigen and to the different reactivity towards complete and incomplete Freund adjuvants of mammals compared with birds. Emulsified vaccines with light mineral oils that, in birds, only exceptionally cause abnormal inflammatory reactions with the formation of granulomas, may cause polyarthritis, amyloidosis, nephrosis, and generalized granulomas in various organs in mammals (Hilleman, 1967). The emulsified vaccines first tested in humans as immunization against influenza and tetanus soon had to be abandoned due to the serious reactions they caused at the point of inoculation (Hilleman, 1967; Pittman, 1967).

The present research on broilers showed the enhancing effect on the immune response to NDV when vitamin E partially replaced light mineral oils in the production of emulsified vaccines. Mineral oils obviously have to be present to a certain extent to induce the inflammation causing the local mobilization of immune cells. In the chicken, vitamin E alone cannot do this; however, when immune-competent cells are found at the point of inoculation, vitamin E enhances their activity.

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