THE INFLUENCE OF CERTAIN SYNTHETIC ESTROGENS ON THE SERUM CALCIUM OF THE CHICKEN

by

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INTRODUCTION

Previous investigations of the calcium content of the blood in the Aves established the fact that the calcium concentration varies in the serum of the female depending upon the activities of the ovaries. In these investigations evidence was presented which showed a relationship between the female estrogenic hormone and blood calcium variation.

Later, a compound was synthesized which was shown to possess estrogenic properties equal to or greater than those of the natural compounds. It affected the serum calcium of chickens in the same manner that the natural compounds did. In addition to its effect on the blood picture, this compound was shown also to possess the ability of increasing the fat content in the various tissues of the bird. Since then, many compounds have been prepared which are capable of producing estrus, and some have been used to fatten fowl, but the effect of these synthetic compounds upon the blood picture of the fowl has not been reported.

This investigation was initiated to determine the effect of some synthetic estrogenic compounds on the serum calcium of the chicken, to determine the quantity of diethylstilbestrol required to produce the calcium effect, to show the effects of high diethylstilbestrol levels and also to demonstrate the effects of prolonged starvation upon the serum calcium of chickens treated with diethylstilbestrol.

SURVEY OF LITERATURE

Riddle and Reinhart (15) have shown that in pigeons the blood calcium was increased to more than twice the normal value at the time the eggs were ready to leave the ovary. The active secretion of an egg shell began approximately 15 hours after the egg left the ovary, however, the beginning of the increase in the concentration of calcium in the blood dated from approximately 108 hours before ovulation or a total of 123 hours before the egg shell began to form. In 1927 Hughes, Titus, and Smits (11) examined the blood of mature hens. Since previous experiments had shown that the amount of calcium and inorganic phosphorous in the blood of normal growing chicks was quite uniform and about the same as that of other normal animals they expected to find a similar uniformity in the calcium content of the blood of the mature hen. Instead of this uniformity, however, they found surprisingly great variations in the calcium content of the hens' blood. They stated that in 10 hens, the values ranged from 13-32 mg per 100 cc of blood. The fluctuation of calcium over a large range was confirmed by Russell, Howard, and Hess (17) who reported a relationship between the blood calcium in the hen and the size of the egg in the ovary. They stated that the presence of ova greater than one centimeter in diameter was accompanied by a blood calcium level between 13.0 and 26.7 mg per 100 cc of serum. The diameter of one centimeter was selected arbitrarily as the dividing line between developing or mature ova and those ova which had not developed or had just started to develop. When

no ova greater than one centimeter were observed, the blood calcium level was between 13.0 and 7.5 mg per 100 cc of serum. Their data indicated, however, that the presence of large ova had high blood calcium did not always indicate active egg production. They concluded by saying, "Whether the development of ova caused the blood calcium to rise or whether an increase in blood calcium stimulated ova formation will have to be investigated, but it should be noted that high blood calcium was never found unless developing ova were present."

Altmann and Hutt (2) in attempting to determine an endocrine basis for the remarkable rise in blood calcium followed the lead of Russell, Howard, and Hess (17) who, as previously noted, showed that the serum calcium rose when an ovum attained a diameter of one centimeter or more. The working hypothesis upon which their investigations were based was, "that something accumulates in the yolk which, upon reaching a certain amount, had the ability to activate the parathyroids and thus to raise the blood calcium." A previous investigator, Fellner (9), demonstrated by testing a lipoid fraction from egg yolk on rabbits that egg contains estrogenic hormone. Allen et al (1) reported negative results with extracts of the hens' eggs. Altmann and Hutt (2) attempted to simulate the laying condition in immature females and in capons by injections of egg yolk and by injections of purified endocrine preparations. They concluded that injections of 50 to 100 cc of egg yolk into the peritoneal cavities of immature female fowls and into the peritoneal cavities of capons induced a significant rise in the level of blood calcium. Similar results were obtained

by the use of the estrogenic hormone in two different preparations of the substance. And removal of yolk from laying hens caused a significant drop in the level of blood calcium, the decrease being directly proportional to the amount of yolk removed. Their operated controls were unaffected, but birds in which the yolk was squeezed from the follicles and left in the body cavity experienced a drop in the serum calcium level to some extent. Since none of the yolk was removed from the body cavity, they believed that its influence on blood calcium was considerably reduced when the yolk was removed from the highly vascularized follicles through which it was ordinarily effective.

Avery, Scott, and Conrad (3) administered larger doses of theelin than did Altmann and Hutt (2) molting hens over a 15 day period without obtaining any significant increase in the blood calcium level. The same results were also obtained in immature pullets when injections were given over a 15 day period, but when the dosage was increased approximately three-fold and injected over a 19 day period, calcium levels were significantly increased. The blood calcium in the experimental birds returned to normal within 6 days when the massive doses of theelin were discontinued.

Landauer et al (13) reported that the serum calcium level of the cocks 6 to 7 months of age varied between 11.0 and 78.0 mg per 100 cc of serum when injected daily with 4,600 I.U. or more of estradiol benzoate. The cocks which received the highest daily amounts of estrogen had extreme lipemia. The blood serum was a deep canary yellow and was very turbid.

In 1938 Dodds et al (7) described the estrogenic activity of certain derivatives of diphenylethane. They demonstrated, in particular, that the introduction of an ethylene linkage between the and carbon atoms of such compounds greatly increased their potency. Their most potent compound was 4:4'-dihydroxy-α, β-diethylstilbene, a derivative of 4:4'- dihydroxystilbene. Its action paralled that of the natural estrogens and it was capable of initiating estrus in ovariectomized rats when given in doses of 0.3 to 0.4 micrograms. Since 4:4'- dihydroxystilbene is the mother substance of a number of estrogenic substances, they suggested that it be termed stilbestrol. However, this term is usually employed by many investigators when they refer to 4:4'-dihydroxy-α,β-diethylstilbene and this latter compound is also referred to as diethylstilbestrol. Comparative tests showed it to be 2 to 3 times as active as estrone.

Zondek and Marx (19) found that injections of estradiol benzoate could induce lipemia in the cock. This condition is normal in the hen during the laying period. One dose of 1 mg of estradiol benzoate was ineffective. However, 2 days' treatment with 2 mg daily did result in lipemia on the third day. After it was learned that the production of lipemia in the cock was due to the specific action of the estrogenic hormone, it was obvious that the question of whether this synthetic substance, which differs chemically from the estrogenic hormone, was able to exert effects similar or dissimilar should be investigated. According to the authors, 0.5 mg of diethylstilbestrol did not increase blood fat whereas one mg gave rise to an increase of blood fat. Zondek and

Marx (20) have produced a calcemia up to 117.0 mg per cent with estradiol benzoate. They found the action of diethylstilbestrol was similar to that of estradiol benzoate. Two days' administration of 4 mg of diethylstilbestrol caused the blood calcium in the cock to rise to 22.6 mg per cent. Six days' treatment with 24 mg of this compound increased the blood calcium to 41.4 mg per cent.

Lorenz (14) has shown that when subcutaneious implants of diethylstilbestrol pellets were made in a group of cockerels 3 weeks of age, each of the implanted birds' tissues had striking and consistently greater fat content than those of controls. This has proved to be of value in the fattening of poultry since diethylstilbestrol is relatively inactive when administered orally. The implantation acts for a long period of time and eliminates the necessity of continually injecting the bird.

Clegg et al (5) showed that the implantation of diethylstilbestrol pellets into immature cockerels gave significant serum calcium rises and also an electrophoretic pattern similar to that of laying hens.

In 1945 Jaap (12) tested several synthetic estrogens and found that triphenylchloroethylene was active in chickens and turkeys when administered via the alimentary tract. The activity was measured by determining the increase in weight of the oviducts. Hixson and Thompson (10) reported that the Oklahoma A. & M. Poultry Department Staff stated that the synthetic estrogen triphenylbromoethylene was of value for fattening turkeys when given orally.

Rinderknecht and Rowe (16) in the course of an investigation on the alkylation of $\alpha:\alpha$diphenylacetophenone obtained a series of compounds which were structurally related to triphenylethylene. These derivatives appeared to be of interest as potential estrogens. They carried out assays on these compounds according to standard procedures and several gave evidence of possessing relatively high potency when compared with theelin.

Recently a compound called ECP which is technically known as estradiol cyclopentylpropionate has been reported (8). It is a derivative of estradiol and has been used successfully for treating temporary sterility in cattle. Correction was found in 93.1 per cent whereas only 58.8 per cent was successful using stilbestrol.

METHODS AND MATERIALS

Samples of blood were obtained from the wing vein. The serum was prepared by mild centrifugation of the blood clot, and the serum calcium was determined by the Clark-Collip (4) modification of the Kramer-Tisdall procedure.

Preparation of Triphenylbromoethylene[1] and Triphenylchloroethylene[1] Pellets

The approximate melting points of the 2 compounds were determined. The melting point of triphenylbromoethylene was found to

1 Supplied by Mallinckrodt Chemical Works

be between 113°-114° C. and the melting point of triphenylchloroethylene was between 115°-116° C. The compounds were then placed in small beakers which were partially submerged in oil baths and the temperature of the oil baths was then raised 5°-10° C. above the melting points of the compounds. When the compounds melted, they were rapidly poured upon an aluminum foil which was placed on top of a porcelain plate. The porcelain plate had small cup-like depressions in it and the aluminum foil was forced into the depressions. The liquified compounds were poured into the depressions. Upon solidification, the substances were separated from the aluminum foil and stored in bottles until used.

Preparation of Triphenylbromoethylene and Triphenylchloroethylene Capsules

Triphenylbromoethylene and triphenylchloroethylene capsules were prepared by filling size no.5 gelatin capsules. Prior to filling, the average weight of 6 capsules was obtained. After the capsules were filled, they were weighed again. The average weight of the empty capsule subtracted from the weight of the filled capsule gave the weight of the compound. Precautions were taken to avoid dermal contact with the compounds since the absorptive capacity of the skin for the compounds was not known. Therefore, rubber gloves were employed when the capsules were filled in order to provide an element of safety.

Preparation of Diethylstilbestrol[2] Solutions

The diethylstilbestrol powder was weighed and transferred to a 25 ml volumetric flask. Then 10-15 ml of propylene glycol was added and the mixture shaken and allowed to stand until solution was complete. Then propylene glycol was added to the mark and the solution was shaken until homogeneity was attained. This procedure was followed only in cases where the final concentration was 5 mg per ml or less. When the concentration was greater, 1-15 ml of propylene glycol was added, then the mixture was heated in a hot water bath at 65°-75° C. until solution was complete. Then propylene glycol was added to the mark and the solution shaken until homogeneity was attained.

Preparation of Triphenylchloroethylene Solution

The compound was weighed and transferred to a 25 ml volumetric flask and 10-15 ml of corn oil was added to the flask. The flask, with its contents, was heated in a Bunsen flame until the compound dissolved. Then the mixture was cooled and sufficient corn oil added to bring it to volume. The flask was then shaken until the solution was uniform.

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[2] Merck & Co., Inc.

Method of Preparing Solutions of α-Methyl-α,α-diphenylacetophenone[3], α-Ethyl-α,α-diphenylacetophenone[3], Triphenylethoxyethylene[3], and Triphenyl-β-N-piperidinoethoxyethylene Hydrochloride[3]

The compounds were weighed and transferred to a volumetric flask as previously described. Fifteen-20 ml of purified peanut oil was added and the flask then heated in a hot water bath between 75°-100° C. When solution was complete in all flasks except the flask containing triphenyl-β-N-piperidinoethoxyethylene hydrochloride, they were cooled and additional solvent was added to dilute it to volume. Since all of the contents in the flask containing triphenyl-β-N-piperidinoethoxyethylene hydrochloride did not dissolve, the solution was assumed to be saturated. It was cooled and solvent was added to bring it to volume. Then each flask was shaken until the solution was uniform.

Estradiol Cyclopentylpropionate[4] (ECP)

Estradiol cyclopentylpropionate was supplied by its producer in soluble form.

Administration of the Various Compounds

The compounds administered in solution form to the chickens were given by intramuscular injection into the thigh. Pelleting

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3 Supplied by H. Rinderknecht
4 Supplied by the UpJohn Company

was done by means of a pellet implanter. This instrument placed the pellets beneath the skin and above the muscle tissue. The pellets were placed in the neck region. The triphenylchloroethylene and triphenylbromoethylene pellets were made as previously described. Capsules of the same compounds were also administered orally. Diethylstilbestrol employed in pellet form was obtained from the manufacturer[5].

EXPERIMENTAL

Relationship Between the Rise of Serum Calcium and the Time of Diethylstilbestrol Pellet Implantation

In the first phase of the investigation, two groups of 5 month old cockerels were subcutaneiously implanted with two 15 mg pellets of diethylstilbestrol. Blood samples were obtained from some birds prior to hormone implantation and these served as controls. The remaining blood samples were obtained on the second, fourth, seventh, and eleventh days in the case of the first group, and blood samples were obtained from the second group on the second, fourth, sixth, and ninth days after treatment. Calcium analyses were performed on the sera. The results are illustrated in Figs. 1 and 2. Plate I is a composite of Figs. 1 and 2. The results of both series are tabulated in Tables 1, 2, and 3. The average calcium value of the untreated birds was 11.1

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[5] Chemi-Ca' Pellets

TRIAL I

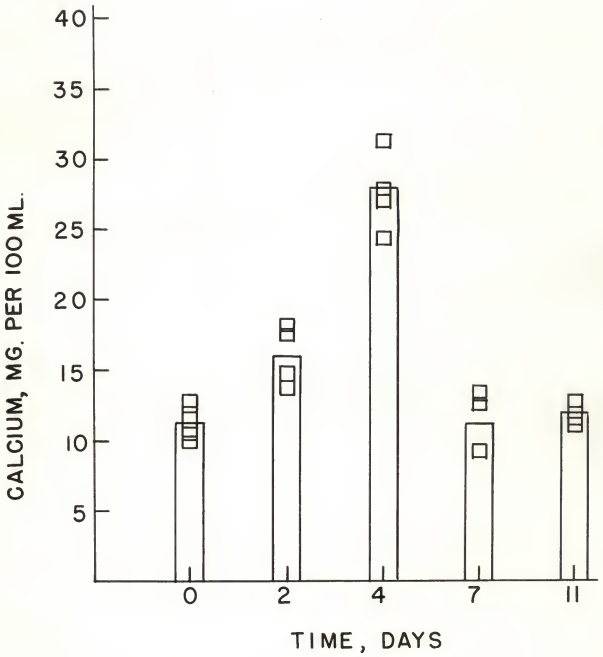

Fig. 1. Relationship between the time of diethylstilbestrol pellet implantation and the rise in serum calcium.

TRIAL I

Table 1. Relation between the rise of serum calcium and the time of diethylstilbestrol pellet implantation.

Days after Implantation	Calcium, mg per 100 ml of serum				
	0	2	4	7	11
	12.8	13.6	31.2	13.1	12.8
	11.4	14.6	26.9	9.3	11.2
	10.1	17.5	27.7	12.5	11.7
	10.4	18.1	24.3	-	11.8
	11.2± 0.9	15.9 ± 1.9	27.5 ± 1.7	11.6 ± 1.7	11.9 ± 0.5

TRIAL II

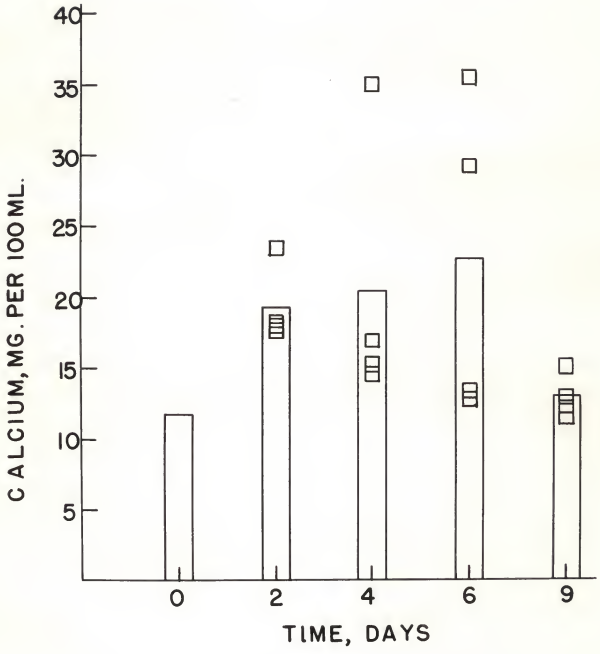

Fig. 2. Relationship between the time of diethylstilbestrol pellet implantation and the rise in serum calcium.

TRIAL II

Table 2. Relation between rise of serum calcium and time of diethylstilbestrol pellet implantation.

Days after implantation	Calcium, mg per 100 ml of serum				
	0	2	4	6	9
	12.8	18.1	35.0	29.3	13.0
	11.4	23.4	14.7	12.8	15.2
	10.1	18.2	15.4	13.3	12.3
	10.4	17.9	17.0	35.4	11.8
	11.2 ± 0.9	19.4 ± 2.0	20.5 ± 7.2	22.7 ± 9.7	13.4 ± 1.1

EXPLANATION OF PLATE I

Composite average for the relationship between the time of diethylstilbestrol pellet implantation and the rise of serum calcium.

PLATE I

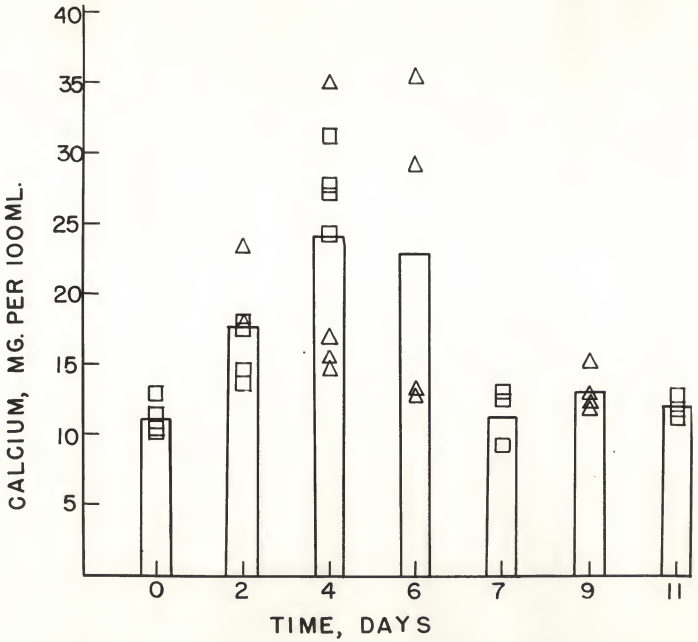

Table 3. Composite average for diethylstilbestrol pellet implantation experiment.

Trial	Days after implantation	Calcium, mg per 100 ml of serum						
		0	2	4	6	7	9	11
1		11.2	15.9	27.5	-	11.6	-	11.9
2		-	19.4	20.5	22.7	-	13.4	-
		11.2	17.7	24.0	22.7	11.6	13.4	11.9

mg per 100 ml of serum. On the second day, the average value rose to 15.9 mg per 100 ml of serum. The fourth day's average value rose to 27.5 mg per 100 ml of serum. This is the value typical of hens in the laying state. By the seventh and eleventh days, the average calcium value had returned to that of the untreated birds. The average results of the second series were similar to those of the first series but the results showed considerable individual variation. On the fourth day, individual results showed one cockerel which had a calcium level in the upper region of the laying hen while the other 3 samples taken that day were on, or slightly above the borderline area between laying and non-laying hens. The individual values of the sixth day showed 2 birds that had serum calcium values in the vicinity of the laying hen while 2 others had values in the region of the control birds. From this, it is quite evident that there was considerable individual variation between birds.

Assay Experiment

With the data from the preceding experiment serving as a guide to indicate when the rise of serum calcium occurred, the probability of "an all or none effect" was investigated. The term "all or none effect" indicates that a certain amount of the compound was needed, or not needed, to produce an elevated calcium level in the serum of the chicken. In the pellet implantation experiment the pellets were not always effective and the quantity

of hormone absorbed was not known. In this series the diethylstilbestrol was administered by intramuscular injection in a solution of propylene glycol, the concentration of which was 2 mg per ml. In the first trial, 0.2-1.0 mg of the drug was administered to 5 groups of 6 week old cockerels. In a second trial, the drug was given in quantities ranging from 0.5-2.5 mg. The concentration of the diethylstilbestrol solution was 5 mg per ml. In this manner, check results could be obtained on some points in the first trial. The same birds that were employed for the first run were used for the second run 3 days after the hormone treatment had been discontinued. A third trial performed, using a different group of 9 week old cockerels instead of 6 week old birds and a diethylstilbestrol solution with concentration of 5 mg per ml, gave essentially the same results as the first and second trials. This trial was designed to determine if a quantity of diethylstilbestrol midway between 1.0 and 1.5 mg would also be effective. During each trial the drug was injected at each level daily for 4 days and blood samples were obtained on the fourth day after the first injection. Calcium determinations were then performed on the serums. The results of all three trials are illustrated in Figs. 3, 4, and 5. Plate II is a composite of the 3 trials. Tables 4, 5, 6 and 7 give the data for Figs. 3, 4, 5 and Plate II.

The average calcium values of the treated birds were between 11.8 and 12.7 mg per 100 ml of serum in the first trial and did not differ significantly from that of the control group which was 12.3 mg per 100 ml of serum. There was no control group used in

TRIAL I

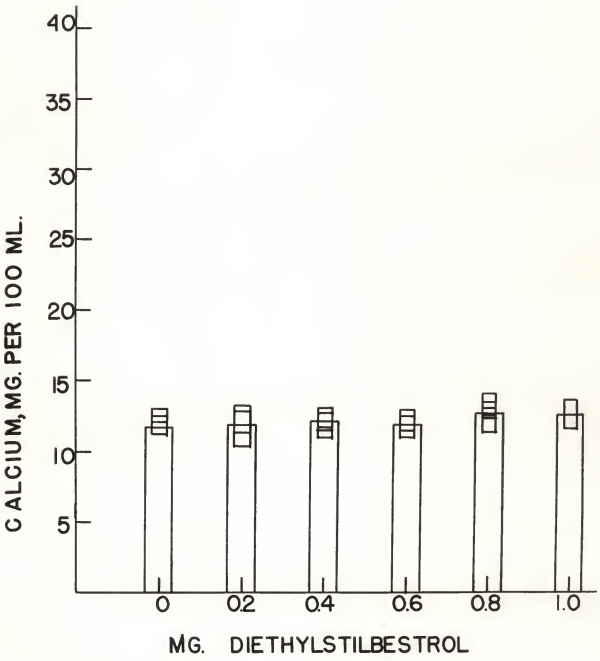

Fig. 3. Relationship between the amount of diethylstilbestrol and the rise in serum calcium.

TRIAL I

Table 4. Assay experiment.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	1	12.2
	2	12.3
	3	12.5
		12.3 ± 0.1
0.2	4	12.2
	5	12.2
	6	10.9
		11.8 ± 0.6
0.4	7	12.2
	8	11.8
	9	12.2
		12.1 ± 0.2
0.6	10	12.3
	11	11.7
	12	11.5
		11.8 ± 0.3
0.8	13	11.8
	14	13.4
	15	13.0
		12.7 ± 0.6
1.0	16	13.1
	17	12.0
	18	-
		12.5

TRIAL II

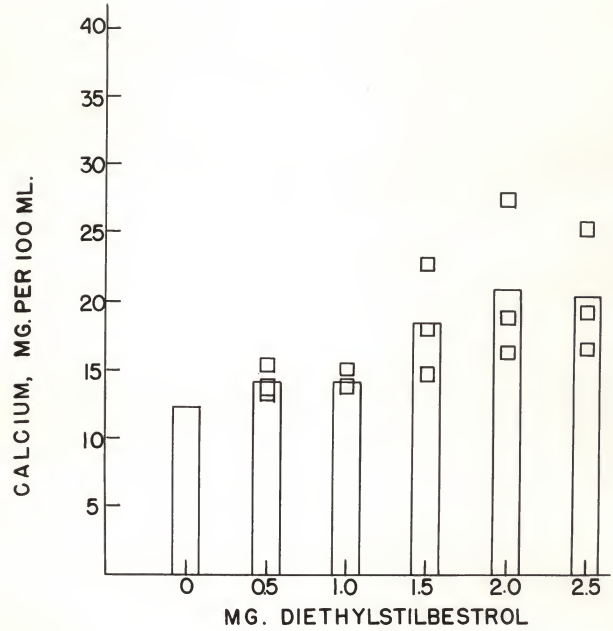

Fig. 4. Relationship between the amount of diethylstilbestrol and the rise in serum calcium.

TRIAL II

Table 5. Assay experiment.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	1	12.2
	2	12.3
	3	12.5
		12.3 ± 0.1
0.5	4	15.4
	5	13.4
	6	13.6
		14.1 ± 0.8
1.0	7	13.8
	8	13.6
	9	15.0
		14.1 ± 0.6
1.5	10	22.7
	11	14.6
	12	18.0
		18.4 ± 2.8
2.0	13	18.7
	14	16.3
	15	27.4
		20.8 ± 4.4
2.5	16	25.3
	17	16.5
	18	19.2
		20.3 ± 3.3

TRIAL III

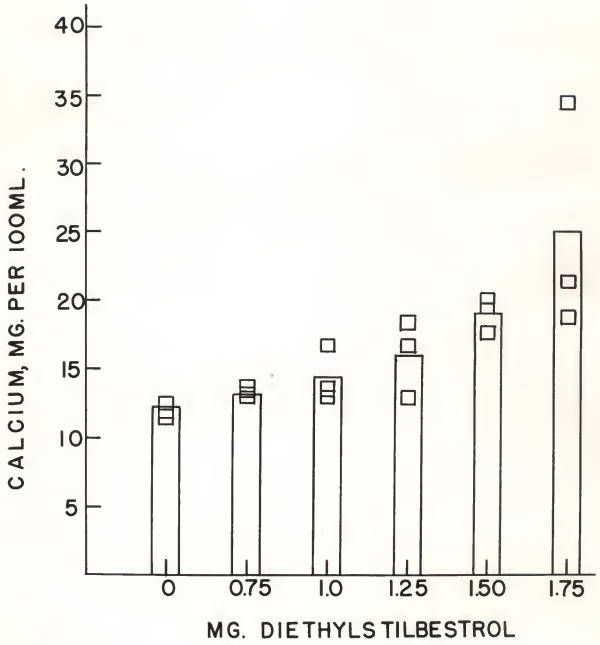

Fig. 5. Relationship between the amount of diethylstilbestrol and the rise in serum calcium.

TRIAL III

Table 6. Assay experiment.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	1	12.5
	2	12.3
	3	12.0
		12.3 ± 0.2
0.75	4	13.3
	5	12.8
	6	13.1
		13.1 ± 0.2
1.0	7	16.6
	8	12.8
	9	13.4
		14.3 ± 1.6
1.25	10	16.6
	11	18.4
	12	12.8
		15.9 ± 2.1
1.5	13	20.0
	14	17.6
	15	19.5
		19.0 ± 1.0
1.75	16	21.8
	17	34.4
	18	18.6
		24.9 ± 6.3

EXPLANATION OF PLATE II

Composite average for the relationship between the amount of diethylstilbestrol and the rise in serum calcium.

PLATE II

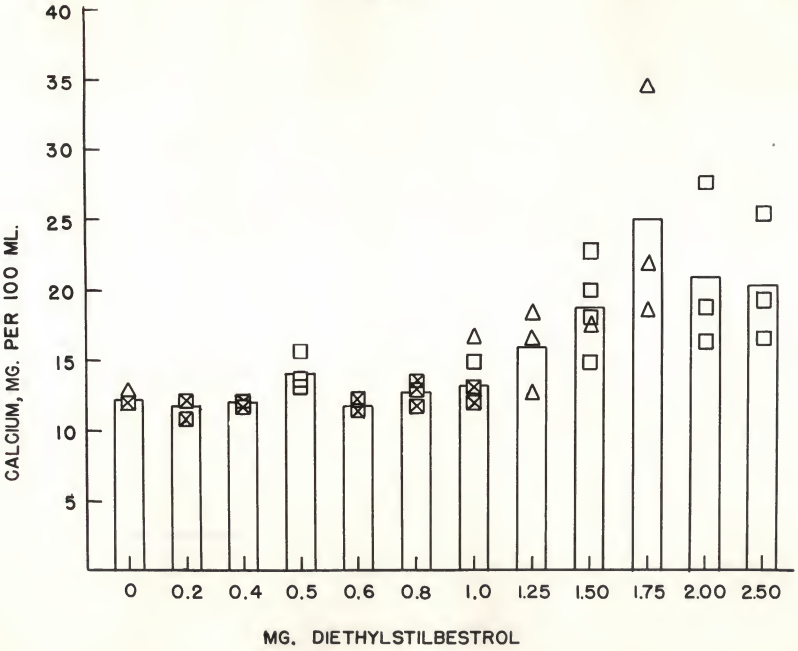
CALCIUM, MG. PER 100 ML.
40
35
30
25
20
15
10
5
0
0.2
0.4
0.5
0.6
0.8
1.0
1.25
1.50
1.75
2.00
2.50
MG. DIETHYLSTILBESTROL

Table 7. Composite average for assay experiment.

Calcium, mg per 100 ml of serum

Trial	Level (mg)	0.0	0.2	0.4	0.5	0.6	0.75	0.8	1.0	1.25	1.5	1.75	2.0	2.5
1		12.3	11.8	12.1	-	11.8	-	12.7	12.5	-	-	-	-	-
2		-	-	-	14.1	-	-	-	14.1	-	18.5	-	20.8	20.3
3		12.3	-	-	-	-	13.1	-	14.3	15.9	19.0	24.9	-	-
		12.3	11.8	12.1	14.1	11.8	13.1	12.7	13.6 ±0.9	15.9	18.7	24.9	20.8	20.3

the second trial. The control group with which it was compared was the control group of the first trial. In this trial, the 0.5 and 1.0 mg doses of diethylstilbestrol gave slightly higher average calcium values of 14.1 mg per 100 ml of serum than similar quantities of the drug in the first trial. However, doses ranging from 1.5-2.5 mg did unmistakably show the calcium effect of the synthetic estrogen. The average calcium values in this region ranged between 18.4 and 20.8 mg per 100 ml of serum. In the third trial, an average calcium value of 13.1 mg per 100 ml of serum was obtained for 0.75 mg of the compound. This value was not shown in Plate II. The 1.25 mg dose of this compound showed a greater average calcium value than quantities ranging from 0.2-1.0 mg. This average was 15.9 mg per 100 ml of serum. The average calcium values produced by the 1.25-1.75 mg of the hormone ranged from 15.9-24.8 mg per 100 ml of serum.

The composite graph of Fig. 7 indicates that a definite calcium effect was obtained when the amount of diethylstilbestrol administered was 1.25 mg or greater.

Effect of Repeated High Estrogen Level

The 5 month old cockerels treated with subcutaneous pellet implantations did not show a sustained calcium elevation after the sixth day, but the fattening effect of the synthetic estrogen continues for several weeks. Investigations of the effect of large amounts of the compound were undertaken as follows. A

group of sixteen 4 month old cockerels was treated daily by intramuscular injection with 20 mg of diethylstilbestrol in propylene glycol. The concentration of the diethylstilbestrol solution was 40 mg per ml. Eight were treated for 10 days and the remainder were treated for 11 days. In the group that was treated for 10 days, blood samples were obtained from some birds on the seventh, fourteenth, and twenty-fifth days after the injections were discontinued. In the group that was treated for 11 days, blood samples were obtained from some of these birds on the sixth, thirteenth, and twenty-fourth days after treatment was halted. If the numbers just given were calculated from the day after the first injection, the sixth and seventh days, the thirteenth and fourteenth days, and the twenty-fourth and twenty-fifth days would coincide since treatment for all of the birds began at the same time whereas one group of birds was treated 1 day longer than the other group. The results are summarized in Table 8. When compared with control samples taken at random throughout the experiment, the serum calcium showed a rise on the first day after the initial injection. The serum calcium continued to rise in most birds throughout the injection period. On the first day after the initial injection, the average calcium value was 16.9 mg, on the second day, 30.7 mg, on the fourth day, 69.7 mg, on the sixth day, 98.3 mg, on the seventh day, 105.0 mg, on the ninth day 101.2 mg, and on the eleventh day 111.2 mg per 100 ml of serum. On the eleventh day, the individual serum calcium levels varied from 88.3 to 131.2 mg per 100 ml of serum. The average calcium values were still high on the sixth and seventh days after the injections had

Table 8. Effect of repeated high estrogen level.

Bird	Calcium, mg per 100 ml of serum											
	Samples during injection period							Days after injections discontinued				
	Days after first injection											
	1	2	4	6	7	9	11	6	7	13	14	24	25
							Non-treated						
17 7897	-	-	12.5	-	-	-	12.8	-	-	-	-	-	14.2
18 white rooster	-	-	13.1	-	-	-	-	-	-	-	-	-	13.8
19 9990	-	-	10.2	-	-	-	-	-	-	-	-	-	12.2
20 9994	-	-	-	14.4	-	-	-	-	-	-	-	-	12.8
			11.9 ±1.2	14.4			12.8						13.3 ±0.8
							Treated						
1 G93	17.6	34.2	93.1	96.0	128.0	-	112.6	-	-	-	-	-	12.8
2 Y17	18.1	35.2	67.2	96.0	95.4	95.0	100.8	-	-	62.4	-	13.1	-
3 B92	16.2	28.8	67.2	-	-	-	-	81.9	-	-	13.8	-	12.2
4 G91	15.7	25.6	64.0	92.2	-	95.4	104.0	-	-	-	-	32.3	
5 R92	-	29.9	-	-	-	82.8	88.3	-	-	-	-	-	12.6
6 C9992	-	-	52.8	-	108.1	-	126.4	-	-	-	-	-	-
7 Y91	-	-	80.0	-	-	104.0	112.6	-	-	-	-	-	-

Table 8. (concl.)

Bird	Calcium, mg per 100 ml of serum												
	Samples during injection period							Days after injections discontinued					
	Days after first injection												
	1	2	4	6	7	9	11	6	7	13	14	24	25
						Treated							
8C9996	-	-	67.5	-	-	105.9	-	-	-	-	-	13.4	-
9C9993	-	-	65.6	-	102.4	-	120.0	-	-	-	-	-	13.1
10 Y51	-	-	-	107.2	-	-	104.8	-	-	-	-	13.4	-
11 Y58	-	-	-	98.2	-	124.2	131.2	-	113.3	32.0	-	13.4	-
12 Y25	-	-	-	102.6	-	-	-	-	-	-	-	-	13.9
13 Y62	-	-	-	96.0	-	-	-	64.0	-	-	-	-	-
14C9985	-	-	-	-	101.4	-	-	-	-	-	-	12.6	-
15C9995	-	-	-	-	94.4	-	-	-	-	-	-	-	-
16 Y69	-	-	-	-	-	-	-	-	60.2	14.7	-	12.5	-
	16.9 ±1.0	30.7 ±3.2	69.7 ±8.5	98.3 ±3.8	105.0 ±8.9	101.2 ±10.2	111.2 ±10.4	73.0 ±8.3	86.8 ±26.5	36.4 ±17.4	13.8	15.5 ±4.7	12.7 ±0.3

been discontinued with values of 73.0 and 86.8 mg per 100 ml of serum, respectively. The thirteenth day showed a sharp decrease with an average calcium value of 36.4 mg per 100 ml of serum. All but 1 of the individual calcium values were in the region of the untreated birds, hence, the average calcium values of the fourteenth and twenty-fifth days were in the neighborhood of the average values of the control birds. The average value of the twenty-fourth day was somewhat high since 1 of the birds in this group had a high serum calcium. The serum prepared from the blood samples taken on the first and second days was normal in appearance even though the calcium rose during this period. However, the serum prepared from the fourth day on was very yellow and turbid.

This experiment demonstrated that repeated injections with a high level of estrogen could maintain an elevated serum calcium value and cause it to rise greatly above that normally found in the laying hen.

Effect of Single Doses of Diethylstilbestrol

Four groups of 7 week old cockerels 5 in each group, were given a single dose of diethylstilbestrol ranging from 4 to 40 mg and a fifth group of 10 week old cockerels was injected with a single dose of 60 mg. There was also a 7 week old control group of 7 birds. Those receiving the 4 and 10 mg doses received a solution of the diethylstilbestrol containing 10 mg per ml. When the estrogen was given in quantities of 20 and 60 mg it was

applied in a solution of propylene glycol having a concentration of 40 mg per ml. Samples of blood were obtained from all groups on the fourth day after the first single injection of the drug and again on the sixth day for those groups receiving 20 mg or more and then only from 3 out of the 5 birds in each group taken at random. The data is summarized in Table 9. There was no response on the fourth day from those birds receiving the 4 mg quantity of the hormone. The average serum calcium value in this group was 12.9 mg per 100 ml of serum. Those receiving the 10 mg dose showed only 1 bird responding with a calcium value of 25.0 mg per 100 ml of serum. The remainder were in the vicinity of the control birds. The average calcium value was 15.6 mg per 100 ml of serum. At the 20 mg level 3 out of the 5 birds responded, giving an average calcium value of 29.2 mg per 100 ml of serum. At the 40 and 60 mg levels, all the birds responded with average calcium values of 44.7 and 73.6 mg per 100 ml of serum, respectively. For the sixth day, the birds receiving 20 mg of the compound showed decreases in the cases where the 2 values had been high on the fourth day from 41.8 to 12.5 mg and 25.6 to 12.5 mg per 100 ml of serum. The average calcium value was 12.8 mg as compared with 29.2 mg per 100 ml of serum on the fourth day. All samples taken on the sixth day from birds receiving 40 mg of the compound showed decreases in the calcium levels and the average calcium level was 21.8 mg as compared with 44.7 mg per 100 ml of serum for the fourth day. Samples taken on the sixth day from birds receiving 60 mg of the drug showed increases in every case as compared with samples obtained on the fourth day with average calcium values of

Table 9. Effect of a single injection of diethylstilbestrol.

Level (mg)	Bird	Calcium, mg per 100 ml of serum	
		Days after first injection	
		4	6
0.0	26	12.5	-
	27	12.8	-
	28	13.1	-
	29	12.8	-
	30	-	-
	31	-	11.5
	32	-	11.8
		12.8 ± 0.2	11.6
4.0	1	12.5	-
	2	12.8	-
	3	12.8	-
	4	13.1	-
	5	13.1	-
		12.9 ± 0.2	
10.0	6	13.4	-
	7	12.5	-
	8	14.1	-
	9	13.4	-
	10	25.0	-
		15.6 ± 3.7	

Table 9. (concl.)

Level (mg)	Bird	Calcium, mg per 100 ml of serum	
		Days after first injection	
		4	6
20.0	11	13.1	13.4
	12	12.8	-
	13	41.8	12.5
	14	25.6	12.5
	15	52.5	-
		29.2 ± 14.4	12.8 ± 0.4
40.0	16	60.8	-
	17	52.8	39.0
	18	38.7	14.1
	19	22.4	12.2
	20	48.6	-
		44.7 ± 11.8	21.8 ± 8.2
60.0	21	86.4	-
	22	68.8	74.6
	23	64.0	72.0
	24	81.6	83.5
	25	67.2	-
		73.6 ± 8.3	76.7 ± 4.5

76.7 and 73.6 mg per 100 ml of serum, respectively.

The data from Table 9 indicates that a single injection of the compound at a level of 20 mg or more can mobilize the serum calcium by the fourth day. By the sixth day, the serum calcium levels are returning to normal or have already reached the pre-injection value in birds that received 20 and 40 mg quantities of the compound, but birds having received 60 mg of the drug, continued to manifest a further increase in their serum calcium level.

The sera obtained on the fourth day from birds 1 to 12, 14, 19, 26 to 32 appeared normal, that obtained from birds 13 and 15 to 20, excluding number 19, and 21 to 25 had a deep canary yellow color and were turbid. On the sixth day, the sera from birds 13, and 17 to 18 were normal, but that from birds 22 to 24 had a deep canary yellow color and were turbid.

Repelleting as a Means of Maintaining the Serum Calcium Level

An attempt was made also to maintain the increased serum calcium level by repelleting. Four 5 month old cockerels were implanted with two 15 mg pellets of diethylstilbestrol. Prior to implantation, blood samples were obtained from 3 birds to serve as controls. Four days after the initial implantation, blood was again obtained from these birds. Only 1 bird out of the 3 showed an elevated serum calcium. The birds were repelleted 9 days after the first treatment and blood was obtained from the 3 birds 2 days after this. On the eleventh day after the initial im-

plantation, the bird showing an increased serum calcium level after the first implantation also exhibited an elevated serum calcium level after the second implantation. There was also another bird manifesting an augmented serum calcium level, but no blood samples had been obtained from this bird previously, consequently, it is not known if the increase in the serum calcium level was also evident 4 days after the initial implantation. The data is summerized in Table 10 and indicates that repelleting will maintain the serum calcium.

Table 10. Experiment to maintain the serum calcium level by repelleting.

Bird	Calcium, mg per 100 ml of serum			
	No. of times pelleted	0	1	2
1 6621		13.6	17.6	17.6
2 7877		12.2	13.6	12.5
3 213		11.8	13.6	-
4 6846		-	-	22.6
		12.5 ±0.7	14.9 ±1.8	17.6 ±3.4

First Starvation Experiment with Pellets

Four 5 month old cockerels were taken off feed and on the fourteenth day of starvation 2 of them were pelleted with two 15 mg pellets of diethylstilbestrol and the other 2 served as con-

trols. Blood samples were obtained on the second and third days after implantation. The data is summarized in Table 11. The average serum calcium values of the control birds for the second and third days were 11.7 and 12.0 mg per 100 ml of serum, respectively. The average serum calcium values of the treated birds for the second and third days were 12.8 and 11.2 mg per 100 ml of serum, respectively. The average serum calcium values of the treated birds did not differ significantly from the average values of the control birds.

Table 11. First starving bird experiment with pellets.

Bird	Calcium, mg per 100 ml of serum	
	Starved 2 weeks. Samples taken days after implantation	
	2	3
	Non-treated	
1 6728	13.9	13.0
2 6066	9.4	11.0
	11.7	12.0
	Treated	
3 3011	-	10.9
4 8272	12.8	11.4
	12.8	11.2

Relationship Between Starvation and Increase in Serum Calcium

Six 6 month old cockerels were taken off feed. On the eighth, sixteenth, and twenty-second days of starvation 2 birds were pelleted with two 15 mg pellets of diethylstilbestol. Samples of blood were obtained 4 days after pellet implantation and calcium determinations were made on the sera. The data is summarized in Table 12. The average calcium value for the birds starved 8 days was 19.2 mg per 100 ml of serum. This differed significantly from control birds in other experiments. For birds starved 16 days, the average calcium value was 14.1 mg per 100 ml of serum. This was not considerably different from untreated birds in previous experiments. The birds starved for 22 days, died before blood samples could be obtained.

This experiment indicated that after birds are starved for a certain period of time, diethylstilbestrol is no longer able to effect an increase in the serum calcium of the chicken. It has been shown that pellets of diethylstilbestrol may not produce the calcium effect under normal conditions, hence, the fact that no rise in serum calcium occurred after 16 days of starvation may not be a result of food deprivation, but it may be a result of failure of the pellets to take effect.

Table 12. Relation between starvation and rise in serum calcium.

Days starved prior to diethylstilbestrol implantation	Calcium, mg per 100 ml of serum		
	8	16	22
	17.6	13.8	-
	20.8	14.4	-
	19.2	14.1	

Starvation Experiment with Injections

A group of eleven, 23 week old birds was starved for 7 days. Three were selected at random and treated intramuscularly for 4 days with 2 mg of diethylstilbestrol in a solution of propylene glycol having a concentration of 10 mg per ml. Blood samples were taken on the fourth day after the first injection. The serum from one bird had entirely coagulated, and therefore, was not available for the calcium determination. Of the remaining two samples, one value was up to 29.3 mg and the other value was unaffected at 12.5 mg per 100 ml of serum. Fourteen days after starvation had begun, 6 birds were treated with the hormone intramuscularly for 4 days and samples of blood were obtained 4 days after the initial injection. The individual serum calcium values were up in every case. The same procedure was repeated 19 days after starvation on 3 birds. The individual serum calcium values in 2 birds were higher than in the controls. However, one of the values was in the vicinity of the control birds. On the twentieth day, the birds

which remained were placed on a diet of cerelose to assist them in surviving until the last blood samples were taken. On the twenty-first day, these birds were treated with the drug as previously described. The individual serum calcium values at this time were higher than controls, but in every case where an earlier value existed for the same bird, the serum calcium value obtained after the twenty-first day of starvation was lower than the earlier value. Some untreated birds served as controls. The data, summarized in Table 13, indicates that after twenty-one days of starvation, the serum calcium level is not affected to the same extent by diethylstilbestrol treatment as it is during the earlier period of starvation.

Synthetic Compounds other than Diethylstilbestrol

Other synthetic compounds have been reported which possess estrogenic activity. Among them are triphenylchloroethylene and triphenylbromoethylene. In addition to their estrogenic property, these compounds have been employed with positive results in feed to fatten fowl (chickens, turkeys).

Nothing has been said concerning their ability to affect the serum calcium of the chicken. Since they have been shown to possess oral activity, some chickens were fed the compounds in capsule form. In addition, the compounds were also injected and implanted.

Several other compounds related to triphenylchloroethylene and triphenylbromoethylene have been shown to possess estrogenic

Table 13. Starvation experiment with injections.

Bird	Calcium, mg per 100 ml of serum			
	Days after starvation injections began	7	14	19	21
		Non-treated			
1 9990		-	11.4	-	-
2 9994		-	11.4	11.4	12.5
3 7897		-	12.2	12.3	13.4
4 white rooster		-	12.2	-	-
			11.8 ±0.4	11.9	13.0
		Treated			
1 G93		-	23.0	-	17.3
2 Y17		29.3	-	-	19.5
3 Y51		12.5	-	-	-
4 R92		-	23.7	-	16.3
5 9985		-	25.9	-	19.5
6 Y69		-	25.6	-	21.3
7 B92		-	26.2	-	20.8
8 Y25		-	18.7	-	17.9
9 Y58		-	-	15.2	-
10 G91		-	-	19.2	-
11 9986		-	-	13.9	-
		20.9	23.9 ±2.1	16.1 ±2.1	18.9 ±1.8

activity. They are ∝methyl-∝,∝-diphenylacetophenone, ∝-ethyl-∝,∝-diphenylacetophenone, triphenylethoxyethylene, and triphenyl-β-N-piperidinoethoxyethylene hydrochloride. It was also of interest to see if these compounds were able to affect the serum calcium of the chicken.

Triphenylbromoethylene Capsule Experiment. Triphenylbromoethylene, in capsule form, was administered daily for 4 days to 2 groups of 8 week old cockerels. One group received approximately 100 mg and the other group 200 mg of the compound. Blood samples were taken on the fourth day after the first capsule was given and calcium determinations made on the sera. The results are summarized in Table 14. The individual and average calcium levels of the treated birds show no significant differences from the control or untreated birds.

Triphenylchloroethylene Capsule Experiment. Triphenylchloroethylene was administered in capsule form each day for 4 days to 2 groups of 3 each of 8 week old cockerels. One group received approximately 100 mg and the second group 200 mg of the compound. Samples of blood were obtained on the fourth day after the first capsule was given and calcium determinations were made on the sera. The results are summarized in Table 15. The individual and average serum calcium levels show no significant differences from control birds.

Triphenylchloroethylene Injection Experiment. Triphenylchloroethylene was administered in a corn oil solution containing 4 mg per ml at a level of 1 and 2 mg to 2 groups of 3 each 9 week old cockerels. The compound was injected intramuscularly for 4

Table 14. Triphenylbromoethylene fed in capsules.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	7	13.3
	8	12.6
		13.0
97.6	1	13.3
99.1	2	14.0
107.5	3	12.0
		13.1 ± 0.7
207.5	4	13.0
119.5	5	13.0
225.6	6	13.1
		13.0 ± 0.0

Table 15. Triphenylchloroethylene fed in capsules.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	7	13.4
	8	12.6
		13.0
116.0	1	13.4
119.9	2	12.5
119.9	3	11.2
		12.4 ± 0.8
244.2	4	12.3
244.0	5	14.0
240.0	6	12.0
		12.8 ± 0.8

days and blood samples were obtained 4 days after the initial injection and calcium analyses were made on the sera. The data is summarized in Table 16. The individual and average serum calcium levels do not differ significantly from the controls with which they are compared.

Triphenylbromoethylene Pellet Experiment I. Pellets of triphenylbromoethylene were implanted in three 13 week old cockerels. The quantity of the compound administered was not determined, but it was approximately half of that employed in the second trial. Blood samples were drawn on the third, seventh, and eleventh days after implantation and analyses for calcium were made on the sera. The results are summarized in Table 17. There were no significant differences observed between treated and untreated birds.

Triphenylbromoethylene Pellet Experiment II. In this experiment, the average level of the compound administered by implantation was 53.5 mg. The drug was given to three 14 week old cockerels and blood samples were taken on the fourth and seventh days after implantation and calcium determinations were run on the sera. The results are summarized in Table 18. No significant differences were observed between treated and untreated birds.

Triphenylchloroethylene Pellet Experiment I. Triphenylchloroethylene pellets were implanted in three 13 week old cockerels. The amount of the substance administered was not determined, but it was approximately half of that employed in the second trial. Samples of blood were obtained on the third, seventh, and eleventh days after implantation and calcium determinations were made on

Table 16. Triphenylchloroethylene injected in corn oil.

Level (mg)	Bird	Calcium, mg per 100 ml of serum
0.0	7	12.5
	8	12.3
	9	12.0
	10	12.5
		12.3 ± 0.2
1.0	1	12.3
	2	12.0
	3	12.3
		12.2 ± 0.1
2.0	4	12.3
	5	13.1
	6	12.5
		12.6 ± 0.3

Table 17. Triphenylbromoethylene pellet experiment I.

Bird	Days after implantation	Calcium, mg per 100 ml of serum		
		3	7	11
		Non-treated		
4		-	-	13.0
				13.0
		Treated		
1		12.6	12.5	12.5
2		11.1	11.7	11.7
3		12.5	12.0	12.3
		12.1 ± 0.6	12.1 ± 0.3	12.2 ± 0.3

Table 18. Triphenylbromoethylene pellet experiment II.

Level (Average mg)	Bird	Calcium, mg per 100 ml of serum	
		Days after implantation 4	7
0.0	4	-	12.6
			12.6
53.5	1	12.5	13.3
	2	13.0	12.5
	3	12.6	13.0
		12.7 ±0.2	12.9 ±0.3

the sera. The results are summarized in Table 19. There were no significant differences between treated and untreated cockerels.

Triphenylchloroethylene Pellet Experiment II. For this experiment, the average dosage of the drug administered by implantation was 47.0 mg. The compound was given to three 14 week old cockerels and blood samples were procured on the fourth and seventh days after treatment. Then calcium determinations were made on the sera. The results are summarized in Table 20. There were no significant differences between treated and untreated birds.

α-Methyl-α,α-diphenylacetophenone. Three birds were treated with α-methyl-α,α-diphenylacetophenone which was dissolved in peanut oil. The concentration of the solution was 5.6 mg per ml. The first 2 birds received 11.2 mg of the compound for 3 days

Table 19. Triphenylchloroethylene pellet experiment I.

Bird	Calcium, mg per 100 ml of serum		
	Days after implantation		
	3	7	11
	Non-treated		
4	-	-	13.0
			13.0
	Treated		
1	12.2	11.8	-
2	11.8	12.5	12.6
3	11.8	12.5	12.3
	11.9 ± 0.2	12.3 ± 0.3	12.5 ± 0.1

Table 20. Triphenylchloroethylene pellet experiment II.

Level (Average mg)	Bird	Calcium, mg per 100 ml of serum	
		Days after implantation	4	7
0.0	4		-	12.6
				12.6
47.0	1		12.3	11.5
	2		12.2	12.0
	3		12.2	12.2
			12.2 ±0.0	11.9 ±0.0

and 25.2 mg apiece on the fourth day. The third bird received 5.6 mg per day for 4 days. Blood samples were taken on the fourth day after the initial injection and calcium determinations were made on the sera. The results are summarized in Table 21. The individual and average serum calcium values of the treated birds did not differ from the control birds.

∝-Ethyl-∝,∝-diphenylacetophenone. Three birds were treated with a solution of 2.5 mg of∝-ethyl-∝,∝-diphenylacetophenone dissolved in a ml of peanut oil. One bird was treated with 5 mg per day for 3 days and 10 mg on the fourth day. A second bird received 5 mg per day for 3 days and 12.8 mg on the fourth day. The third received 2.5 mg per day for 4 days. Samples of blood were obtained on the fourth day after the first injection and calcium analyses were made on the sera. The results are summarized

in Table 21 and do not show any significant differences between treated and untreated birds.

Triphenylethoxyethylene. Three birds were treated with a solution of 5.2 mg of triphenylethoxyethylene dissolved in a ml of peanut oil. The first bird received 10.4 mg per day for 3 days and 20.8 mg on the fourth day. The second bird received 10.4 mg per day for 3 days and 26.8 mg on the fourth day. The third one received 5.2 mg per day for 4 days. Blood samples were obtained on the fourth day after the first injection and calcium determinations were made on the sera. The results are summarized in Table 21 and show no significant differences between treated and untreated birds.

Triphenyl-β-N-piperidinoethoxyethylene Hydrochloride. Two birds were treated daily for 3 days with 2 ml of a saturated solution of triphenyl-β-N-piperidinoethoxyethylene hydrochloride in peanut oil. On the fourth day, each bird received 4.5 ml of the compound. Blood samples were taken on the fourth day after the first injection and calcium analyses were made on the sera. The results, as summarized in Table 21, do not show any significant differences between treated and control birds.

Estradiol Cyclopentylpropionate (ECP) Concentration versus Calcium Level. Estradiol cyclopentylpropionate (ECP) has been reported as a potent derivative of estradiol. This might be called a synthetic estrogen, but according to a review by R.S. Teague (18) the term synthetic estrogen includes, "those compounds which have estrogenic activity and yet lack the cyclopentanophenanthrene nucleus. This definition excludes the derivatives of the natural

Table 21. Synthetic hormone experiment.

Total level (mg)	Birds	Calcium, mg per 100 ml of serum
	Non-treated	
0.0	12	11.4
	13	10.9
	14	12.5
		11.6 ± 0.6
	∝-Methyl-∝,∝-diphenylacetophenone	
58.8	1	12.0
58.8	2	12.5
22.4	3	11.5
		12.0 ± 0.3
	∝-Ethyl-∝,∝-diphenylacetophenone	
25.0	4	11.4
27.7	5	12.0
10.0	6	11.5
		11.6 ± 0.2
	Triphenylethoxyethylene	
52.0	7	11.2
58.8	8	11.4
20.8	9	13.6
		12.1 ± 1.0
	Triphenyl-β-N-piperidinoethoxyethylene hydrochloride	
10.5 ml Saturated solution	10	12.2
10.5 ml Saturated solution	11	11.7
		12.0

estrogens from consideration, although strictly speaking, such a compound as ethinyl estradiol is a synthetic substance and does not occur as such in nature." The effect of ECP upon the serum calcium of the chicken was investigated.

Two 12 week old cockerels were each injected intramuscularly with 2 mg per day of estradiol cyclolentylpropionate for 3 days and on the fourth day, each bird received 2.8 mg of this compound. The concentration of the solution containing the drug was 2 mg per cc. Blood samples were obtained on the fourth day after the first injection, and also on the eighteenth day after the last injection. The results are summarized in Table 22. The average serum calcium value for the fourth day was significantly higher than that of the control. For the average serum calcium value obtained eighteen days after the last injection, there was no significant difference between it and that of the control. This shows that estradiol does not lose its ability to elevate the serum calcium when it is esterified with the cyclopentylpropionate group.

Table 22. Estradiol cyclopentylpropionate (ECP) concentration versus calcium level.

Level (mg)	Bird	Calcium, mg per 100 ml of serum	
		Days after first injection	Days after injections discontinued
		4	18
0.0	3	13.0	-
		13.0	
8.8	1	20.5	12.5
	2	39.7	13.1
		30.1	12.8

DISCUSSION

Many workers have shown that the various forms of the natural female estrogen and the synthetic estrogen diethylstilbestrol are able to effect the serum calcium in the fowl. It has been demonstrated also that pellet implants of diethylstilbestrol will cause an increase in the fat content of the birds' tissues and that the fattening effect continues for several weeks.

In the present investigation, cockerels were implanted with pellets of diethylstilbestrol in order to determine where the maximum calcium effect would be observed. According to the data the maximum effect was obtained approximately 4 days after pellet implantation. By the seventh day, however, the serum calcium had returned to the level usually found in untreated cockerels. This indicated that although the absorption from the compound in pellet form might continue in sufficient quantity to cause the deposition of fatty materials in the tissues, the quantity of the compound absorbed beyond the sixth day was insufficient to maintain an elevated serum calcium. There was considerable variation between birds and in many, the pellets failed to exhibit any effect. However, in those birds in which the rise in serum calcium was observed, the above average was noted.

The preceding knowledge was used to determine the minimum quantity of diethylstilbestrol which would cause an elevated serum calcium level. Cockerels were then injected daily with different quantities of diethylstilbestrol and blood samples were obtained at the time the maximum effect should have been evident. Daily

quantities of 1.25 mg or more of the compound over a 4 day period induced calcemia. In general, it appears that a certain quantity of the compound is required to cause an increase in the serum calcium level of the chicken.

In attempting to sustain the elevated serum calcium level beyond the time limit obtained with single implantations, cockerels were repelleted and others also received high levels of diethylstilbestrol by injection. Both of these methods indicated that the elevated serum calcium level could be maintained beyond the period of time obtained by single implantations. The blood serum of the birds treated with high levels of the compound developed also the deep canary yellow color and turbidity reported by Landauer et al (13) when they used estradiol benzoate. Serum calcium levels in these birds were 9 to 10 times higher than those found in controls.

In addition to diethylstilbestrol, several other synthetic compounds which possess estrogenic activity were investigated to determine if they, too, were able to produce calcemia. Of these compounds, triphenylbromoethylene and triphenylchloroethylene have been reported to be active when administered to turkeys and chickens via the alimentary canal. The other compounds were α-methyl-α,α-diphenylacetophenone, α-ethyl-α,α-diphenylacetophenone, triphenylethoxyethylene, and triphenyl-β-N-piperidinoethoxyethylene hydrochloride. Many of these compounds were administered in large quantities by means of pellet implants or by intramuscular injections or both. However, none of these synthetic estrogens, in the quantities given, produced calcemia by these

methods. Although the amounts administered were, in many cases, large, it may be they were not of sufficient quantity to cause calcemia. It is also possible that regardless of the amount given, no serum calcium rise will be observed since they may be incapable of causing the calcium effect. These compounds differ structurally from diethylstilbestrol.

Estradiol cyclopentylpropionate, a derivative of the natural estrogen estradiol, was found to be potent with respect to causing calcemia.

It has been shown previously that the electrophoretic pattern of diethylstilbestrol treated cockerels was similar to the pattern obtained from the serum of laying hens. Clegg (6) has demonstrated that this similarity occurred only when calcemia was present. The possibility that calcemia might be related to the condition of the bird was investigated. In order to alter the birds' condition, they were starved for various intervals of time and serum samples were taken. In general, this work indicates that starvation will not inhibit calcemia, but that it decreases its intensity.

An examination of the chemical formulas of the compounds investigated will reveal that all of those which produce calcemia possess one or more substituent hydroxy groups, whereas those which do not cause the response have none. Future work, employing these triphenyl compounds having substituted hydroxy groups, might disclose a relationship between the calcemia producing property of the compound and the presence and position of the hydroxy group.

SUMMARY

1. Diethylstilbestrol pellet implanted cockerels showed a maximum serum calcium increase approximately four days after the initial implantation. The serum calcium returned to pre-implantation values after the sixth day although the fattening effect of the compound continues for several weeks.

2. The minimum quantity of diethylstilbestrol required to produce an elevated serum calcium in the cockerel was shown to be 5 mg.

3. Cockerels treated daily with high levels of diethylstilbestrol exhibited elevated serum calcium values beyond the sixth day. The serum prepared from blood taken from the fourth day on had a deep canary yellow color and was turbid.

4. Cockerels treated with a single injection of diethylstilbestrol showed no increased serum calcium response when they received 10 mg or less of the compound. At 20 mg or above, the compound produced calcemia and in many cases the deep canary yellow color and turbidity of the serum appeared.

5. There were rises in serum calcium levels in birds that were pelleted and repelleted with diethylstilbestrol. This indicated that repelleting will maintain the increased serum calcium level beyond the sixth day.

6. (a) Cockerels starved for different periods of time and then pelleted with diethylstilbestrol indicated that the compound was unable to produce calcemia after the second week of starvation.

(b) However, cockerels starved for different periods of time and injected with diethylstilbestrol indicated that the compound

could produce high serum calcium levels after the second week of starvation. Samples of blood taken during the third week of starvation, still showed high calcium levels, but in every case where samples of blood were obtained previously, the later serum calcium values were not as high.

7. Cockerels treated with synthetic estrogens other than diethylstilbestrol did not exhibit any calcemia.

8. Estradiol cyclopentylpropionate, a derivative of estradiol, produced calcemia in cockerels.

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THE INFLUENCE OF CERTAIN SYNTHETIC ESTROGENS ON THE SERUM CALCIUM OF THE CHICKEN

by

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The fluctuation of the calcium in the serum of the mature hen has been known for the past quarter of a century. Numerous workers have shown that the various forms of the natural female estrogen and the synthetic estrogen diethylstilbestrol were able to affect the serum calcium in the fowl. It has been demonstrated also that pellet implants of diethylstilbestrol cause an increase in the fat content of the birds' tissues and that the fattening effect continues for several weeks. The present investigation was undertaken to determine the effects of diethylstilbestrol and several other synthetic estrogens on the serum calcium of the chicken.

In the first phase of the investigation, cockerels were injected with two 15 mg pellets of diethylstilbestrol in order to determine where the maximum calcium effect would be observed. According to the data, the maximum effect was obtained approximately 4 days after pellet implantation. By the seventh day, however, the serum calcium had returned to the level normally found in untreated cockerels. This indicated that although the absorption from the compound in pellet form might continue in sufficient quantity to cause the deposition of fatty materials in the tissues, the quantity of the compound absorbed beyond the sixth day was insufficient to maintain an elevated serum calcium. There was considerable variation between birds and in many, the pellets failed to exhibit any effect. However, in those birds in which the rise in serum calcium was observed, the above average was noted.

The preceding knowledge was used to determine the minimum quantity of diethylstilbestrol which would cause an elevated serum calcium level. Cockerels were then injected daily with different quantities of the compound and blood samples were obtained at the time the maximum effect should have been evident. Daily quantities of 1.25 mg or more of the compound over a four day period induced calcemia. In general, it appears that a certain quantity of the compound is required to cause an increase in the serum calcium of the chicken.

In attempting to sustain the elevated serum calcium level beyond the time limit obtained with single pellet implantations, cockerels were repelleted and others also received high levels of diethylstilbestrol by injection. Both of these methods indicated that the elevated serum calcium level could be maintained beyond the period of time obtained by single implantations. In addition, the blood serum of those birds treated with high levels of the compound developed the deep yellow canary color and turbidity reported by others when they used estradiol benzoate. Serum calcium levels in these birds were 9 to 10 times higher than those found in controls.

In addition to diethylstilbestrol, several other synthetic compounds which possess estrogenic activity were investigated to determine if they, too, were able to produce calcemia. Of these compounds, triphenylbromoethylene and triphenylchloroethylene have been reported to be active when administered to turkeys and chickens via the alimentary canal. The other compounds were α-methyl-α,α-diphenylacetophenone, α-ethyl-α,α-diphenylacetophenone, triphenylethoxyethylene, and triphenyl-β-N-piperidinoethoxyethylene

hydrochloride. Many of these compounds were administered in large quantities by means of pellet implants or by intramuscular injections or both. However, none of these synthetic estrogens in the quantities given produced calcemia by these methods. Although the amounts administered were in many cases, large, it may be they were not of sufficient quantity to cause calcemia. It is also possible that regardless of the amount given, no serum calcium rise will be observed since they may be incapable of causing the calcium effect. These compounds differ structurally from diethylstilbestrol.

Estradiol cyclopentylpropionate, a derivative of the natural estrogen estradiol, was found to be potent with respect to causing calcemia.

Previous work has shown that the electrophoretic pattern of diethylstilbestrol treated cockerels was similar to the pattern obtained from the serum of laying hens. This similarity was shown to occur only when calcemia was present. The possibility that calcemia might be related to the condition of the bird was investigated. In order to alter the birds' condition, they were starved for various intervals of time and serum samples were procured. In general, this work indicates that starvation will not inhibit calcemia, but that it decreases its intensity.

It is suggested that there may be relationship between the calcemia producing property of the estrogenic compound and the presence and position of the hydroxy group.