TABLE IV. Effect of Carnitine on Fatty Acid Composition of Mouse Epididymal Fat.*

Group	Fatty acid (mole %)†					
	Palmitic	Oleic	Linolenic	Stearic	Palmitoleic	Myristic
Controls (10)	27.1 ± .6	38.9 ± .6	23.3 ± .1	1.6 ± .1	7.2 ± .6	1.8 ± .2
Carnitine (10)	26.5 ± .6	39.0 ± .5	23.0 ± .2	2.5 ± .2	7.3 ± .6	1.8 ± .2

* 100 mg/kg carnitine daily for 4 wk. Final total body and epididymal pad (wet) weights for controls were: 30 ± 1 g and 241 ± 20 mg; for carnitine-treated mice: 29 ± .8 g and 256 ± 18 mg, respectively.

† Only trace amounts of lauric and linolenic acids were found in all samples.

TABLE V. Carcass Composition of Gold-Thioglucose Obese Mice Given Carnitine.*

Group†	Carcass analysis at 8 wk		
	Lipids (% dry wt)	Protein (% dry wt)	Water, %
Controls (7)	70 ± .9	13.7 ± .2	69.2 ± 1.1
Carnitine (6)	67 ± 1.3	16.1 ± .3	65.8 ± 1.2

* 250 mg/kg/day oral carnitine for 4 wk, 500 mg/kg in divided doses for 4 additional wk.

† In 8 wk, control obese mice each increased in mean wt from 46 ± .9 to 63 ± .8 g; carnitine treated mice increased from 46 ± 1.1 to 58 ± 1.6 g. Food consumption decreased about 15% during the last 10 days of experiment for mice given carnitine.

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Effect of MER-29 on Egg Production in the Chicken.* (27158)

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Several studies in rats and man have conclusively shown that 1-[p-(β-diethylaminoethoxy) - phenyl] - 1 - (p - tolyl) - 2 - (p-chlorophenyl) ethanol (MER-29) causes a depression in serum and tissue cholesterol by inhibiting cholesterol synthesis(1,2), as a result of which 24-dehydrocholesterol (desmosterol) accumulates in serum and tissues(3-5). Despite the regular depression of cholesterol concentration, the effects on total sterol levels vary: the tissue sterols are usually normal, whereas blood sterol concentration may be low(4).

Since the chicken egg may contain as much as 0.25-0.3 g of cholesterol(6), the effect of MER-29 on egg production and egg sterol concentration has been studied in the chicken. The experiments reported here demonstrate that MER-29, when fed in large quantities, results in an almost complete replacement of egg cholesterol by desmosterol and that this

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effect is followed by a complete cessation of egg production.

Procedure. Ten, single-comb, White Leghorn hens, 6 months of age, were weighed, divided into 2 groups of 5 each, and placed in individual cages. The control group was allowed free access to a complete layer mash, and the experimental group was fed the same mash which contained 0.5% MER-29. Eggs were collected daily from each of the hens. Eighteen days after beginning the experiment, MER-29 was removed from the diet of 2 of the experimental animals. At the end of 40 days, representative animals were killed and subsequently autopsied.

Sterol analyses were performed on the eggs collected from the start of experiment until the cessation of egg production from a single hen fed MER-29. The intact egg was weighed, the yolk was separated, weighed, macerated, then added to approximately 150 ml chloroform-methanol (2:1). The mixture was shaken for 10 minutes on an International Bottle Shaker and filtered; the residue was re-extracted with chloroform-methanol, and the filtrates were combined and taken to dryness with heating. The residue was then suspended in benzene, and an aliquot was chromatographed on activated silicic acid-celite (1:1) columns, 20 × 1 cm, as described by Frantz *et al.*(7). The eluates containing the esterified sterols and those containing the free sterols were taken to dryness and saponified overnight at room temperature under nitrogen with 5 ml 20% potassium hydroxide in ethanol. The solutions were then extracted twice with 100 ml petroleum ether, the extracts were backwashed with water, taken to dryness, and dissolved in chloroform.

Cholesterol and desmosterol contents were then determined by a previously described modification(8) of the method of Vanden Heuvel *et al.*(9). Aliquot portions of the chloroform solution were injected into a Research Specialties Co. gas-liquid chromatographic assembly equipped with an ionization detector. Six-foot × 4 mm columns packed with 2% SE 30 silicone-1% neopentyl glycol adipate on Gaschrom P, 60-80 mesh, were used in these experiments, and operating temperatures were as follows: Column, 220°; detector, 230°; outlet 300°; vaporizer, 310°. The argon inlet pressure was 30 psi, and the monitored outflow was 100 ml/minute. The areas recorded under the various peaks were estimated as described by Bartlett and Smith (10). Under the conditions of this experiment the peak appearance time of cholesterol was 34 minutes and of desmosterol was 39 minutes. No other sterol peaks were observed.

Results. The egg production records of chickens fed MER-29 and of a group of normal controls are summarized in Fig. 1. There was no difference in average weight between the 2 groups either at beginning (control 1.5 kg; experimental 1.5 kg), or at end of the experiment (control 1.6 kg; experimental 1.5 kg). Throughout the experimental period average egg production ranged from 0.3-0.8 eggs per day in the control group. However, 12 days after the beginning of MER-29, egg production fell in the treated chickens, and no eggs were produced in the group in which MER-29 was continued from the 22nd day until the end of experiment. In 2 two chickens in which administration of MER-29 was stopped on day 18, egg production did not resume until 12 days later, after which it rapidly returned to normal values.

In Fig. 2 is shown the influence of MER-29 on the egg sterol levels in a single chicken from the beginning of treatment until egg production ceased. During this period the weight of the eggs from this animal decreased from 45.3 g to 36.5 g; however, there was no

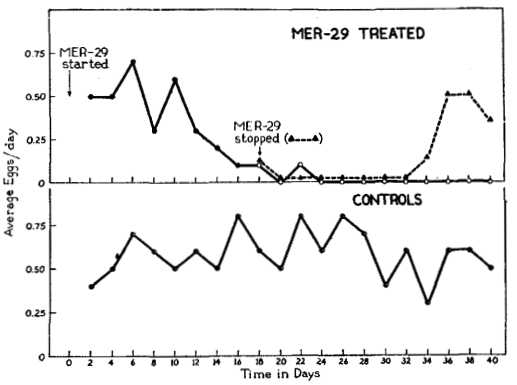

FIG. 1. Influence of MER-29 on avg daily production in the chicken.

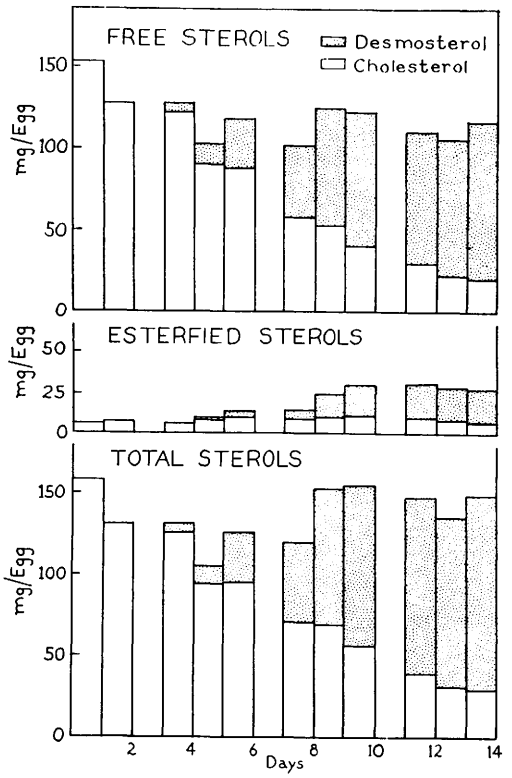

FIG. 2. Effect of MER-29 on sterol levels in the chicken egg.

effect on the weight of the yolk itself (average 11.3 g). During the first 2 days following administration of MER-29 no desmosterol was detected in either free or esterified sterol fractions. On the 4th day, however, a small amount of desmosterol was demonstrable in the free sterols, and desmosterol content increased rapidly thereafter so that by the 14th day about 85% of the total sterol was desmosterol. This almost complete replacement of cholesterol by desmosterol occurred under circumstances in which there was no significant change in total sterol levels in the egg. It is also of interest that the increasing desmosterol concentration did not disrupt the 3-day cycle of egg laying in this hen prior to complete cessation of egg production.

The influence of MER-29 administration on the ovary is illustrated in Fig. 3. The normal ovary weighed 69 g and contained ova in all stages of maturation, whereas the ovary from the MER-29 treated animal weighed 3 g and contained only small, immature ova.

Discussion. This sequence of events strongly suggests that MER-29 inhibits egg production by preventing the onset of maturation of the ovum within the ovary rather than interfering primarily with the subsequent events in formation of the egg. The immature ova of the hen ovary, under the control of follicle stimulating hormone, begin growing at a rapid rate and reach maturity within 9 to 10 days(11); following ovulation there is a delay of about 25 hours before the egg is completed and laid(12). Thus, the time between onset of egg maturation and laying of the completed egg (10-11 days) is quite close to the time intervals required in these experiments for egg production to decrease after onset of MER-29 therapy and to resume after withdrawal of the drug. The conclusion that MER-29 administration inhibits, primarily, maturation of the ovum rather than ovulation or completion of the egg, is also strongly supported by the evidence obtained at autopsy. The ovary from the MER-29 treated chicken contained only small, immature ova and resembled the ovary from an immature hen.

An inhibition of ovum maturation might be due to any of several factors. Popják and Tietz have reported that the membranes surrounding the growing ovum in the chicken

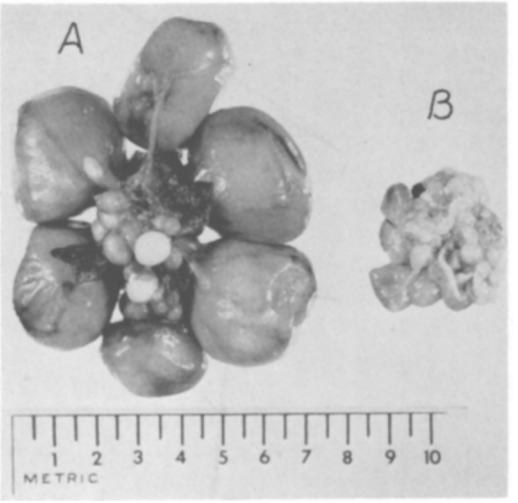

FIG. 3. Influence of MER-29 on the chicken ovary. A. Normal ovary. B. Ovary from MER-29 treated chicken.

ovary are very active in cholesterol biosynthesis, the younger the ova the more active the membranes surrounding them in synthesizing cholesterol(13). The blockage of this synthetic pathway might have deleterious results on ovum maturation if cholesterol is necessary for growth of the ova. It is also possible that desmosterol itself might have some toxic effect on maturation of the ova. Finally it is possible that MER-29, by inhibiting cholesterol synthesis, may interrupt estrogen production in the ovary in a manner similar to its inhibitory influence on steroid synthesis in the adrenal cortex(14); the consequent persistent rise in FSH secretion may then be responsible for cessation of egg laying inasmuch as chronic administration of FSH to laying hens stops egg production(15). The finding in these studies that egg weight (exclusive of yolk), a measure of both ovalbumen and egg shell, fell progressively after institution of MER-29 treatment is suggestive evidence that estrogen production was in fact strongly curtailed since estrogen is known to augment the secretion of ovalbumen (16) and the deposition of the egg shell(17).

Summary. Administration of MER-29 to laying hens is followed over a 2-week period by gradual replacement of 85% of the egg cholesterol by desmosterol. The accumulation of desmosterol in the egg is followed by cessation of egg production; following withdrawal of MER-29 from the diet, egg production returns to normal only after a lag phase of 12 days. This sequence of events suggests that MER-29 inhibits maturation of the ova rather than the subsequent events in egg formation.

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