THE HYPOCHOLESTEROLEMIC ACTIVITY OF ORALLY ADMINISTERED POLYENE MACROLIDES

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In recent studies[1] it has been observed that the oral administration of polyene macrolide antifungal antibiotics to young dogs produced a reduction in the volume of the prostate gland. Likewise, the treatment of old dogs with established natural prostatic glandular hyperplasia resulted in marked gland volume reductions. At the dosage levels employed, the treatment with some polyene macrolides also produced in the prostate gland histological changes that suggest a significant reduction of the hyperplastic condition.

Since these interesting morphological-histological changes in the prostate gland suggested some involvement with steroid hormone metabolism, the possible effect of orally administered polyene macrolides on steroid levels in the dog was investigated. In preliminary studies it was observed that the oral administration of polyene macrolides to dogs markedly influenced the serum cholesterol levels. These studies were greatly enlarged to determine the effect of a variety of polyene macrolides by the oral route on the serum cholesterol levels of the dog. The results are presented in this report.

Materials and Methods.—In these studies a total of 79 purebred beagle dogs (48 males and 31 females), 10–12 months old, weighing approximately 20 pounds were used. The dogs were quarantined for a period of seven days during which time they were stabilized as to diet (Purina dog meal), feeding schedule, and handling.

All polyene antifungal antibiotics employed in these studies were assayed for microbiological potency as determined against *Saccharomyces cerevisiae* ATCC 9763 by a tube dilution procedure.[2] The polyene macrolides employed in these studies included candicidin (S. B. Penick, lot 8031-LHO-11, 45%), amphotericin B (E. R. Squibb, lot 38675-001, 90.4%), filipin (Upjohn, lot U5956, 96%), and nystatin (E. R. Squibb, lot 36982-013, 4230 units/mg). The compounds were placed in hard gelatin capsules and were administered orally with the normal diet, in divided doses given in the morning and late afternoon.

Serum cholesterol levels were determined prior to drug administration. Blood samples were taken daily from the jugular vein on three successive days to determine base line serum cholesterol values. The methods of Leffler[3] and Ferro and Ham[4] were used in these determinations. After 21 days of successive daily drug administration, the animals were finally bled, and the serum cholesterol levels were again determined.

Results.—A summary of the results obtained with the measurement of serum cholesterol levels of dogs after the oral administration of four polyene macrolide antifungal antibiotics is given in Table 1. The heptaene macrolide candicidin[5] was administered at three dose levels, corresponding approximately to 5, 10, and 20 mg/kg body weight. Some gastrointestinal disturbances were noted in the dogs treated with candicidin at the highest dose level. However, discontinuation of the treatment at this dosage level was not necessary. The largest group of dogs (22 animals, 11 female and 11 male) was given candicidin at a single dose level of 5 mg/kg, a dosage level which was comparatively well tolerated. Although the male dogs exhibited a slightly elevated mean cholesterol level prior to

TABLE 1. *Effect of polyene macrolide antifungal antibiotics on serum cholesterol levels in the dog after oral administration.*

Compound tested	Total daily dose (mg)	Number of dogs	Serum Cholesterol Levels Before treatment (mg %)	After treatment (mg %)	Per cent change
Control	150	6	171 ± 30	180 ± 43	+4 ± 8
Candicidin	50	22	174 ± 27	109 ± 22*	−36 ± 14
	100	3	200 ± 35	143 ± 23	−28 ± 3
	200	12	182 ± 18	143 ± 30	−21 ± 12
Amphotericin B	50	3	228 ± 19	174 ± 14	−23 ± 10
	100	3	244 ± 32	163 ± 9	−32 ± 5
	200	3	268 ± 38	166 ± 6	−37 ± 8
	400	3	317 ± 57	169 ± 10	−45 ± 7
Filipin	50	3	275 ± 41	167 ± 14	−38 ± 9
	100	3	296 ± 23	175 ± 9	−40 ± 7
	200	3	333 ± 36	165 ± 9	−50 ± 5
	400	3	231 ± 63	149 ± 9	−31 ± 16
Nystatin	50	3	194 ± 27	234 ± 63	+22 ± 31
	100	3	235 ± 53	188 ± 29	−18 ± 15
	200	3	194 ± 15	186 ± 25	−3 ± 18
	400	3	208 ± 54	230 ± 38	+13 ± 10

Lactose was used as a control. Values given are means and standard deviations.
* Significant difference between the initial and terminal means of the treated group at $p < 0.001$.

treatment, the final cholesterol levels in both males and females were essentially the same. Hence, in these studies no attempt was made to separate the data by sex. The average per cent decrease in serum cholesterol levels (−36 ± 14) after the oral administration of a total daily dose of 50 mg (5 mg/kg) of candicidin is significant ($p < 0.001$). Higher doses of candicidin did not increase the reduction in serum cholesterol levels. This may be due in part to the phenomenon of hemoconcentration associated with the gastrointestinal disturbances which were frequently evident. At the highest dose level these disturbances included emesis as well as loss of appetite and vigor.

Amphotericin B,[6] a heptaene; filipin,[7] a pentaene; and nystatin,[8] a tetraene macrolide were all administered orally at dose levels corresponding to 5, 10, 20, and 40 mg/kg body weight. All of these polyene macrolides were well tolerated at every dosage level, and no signs of gastrointestinal disturbances were evident. After treatment with amphotericin B or filipin at all dosage levels, marked serum cholesterol level reductions were observed. Since the means of the serum cholesterol levels prior to treatment with these substances increased with each increasing dose level administered, it is difficult to ascertain clearly whether or not there is a dose response relationship. The results obtained with nystatin are indeed very different from those obtained with candicidin, amphotericin B, and filipin. A marked variation in serum cholesterol levels is noted, but no clear-cut hypocholesterolemic activity can be attributed to nystatin administered orally in hard gelatin capsules.

It was apparent that the base line serum cholesterol levels, established prior to treatment, varied greatly for the numerous dogs used in these studies. In the course of arranging our experiments, we grouped the dogs according to their base line cholesterol levels. The activity of these polyene macrolides on prostatic

glandular hyperplasia as observed in other studies[1] indicated clearly that these compounds were not of equal potency. Candicidin was found to be most active. From preliminary studies it also appeared that candicidin exhibited very potent hypocholesterolemic activity. The animals treated with candicidin were generally selected among those exhibiting lower base line serum cholesterol levels, whereas those dogs exhibiting higher base line levels were treated with amphotericin B and filipin. Nystatin was administered to dogs exhibiting more intermediate base line cholesterol levels.

From the examination of the results in Table 1 it is very difficult to draw conclusions concerning the relationship of the hypocholesterolemic effect of the polyene macrolides employed and the dose administered. Although the dogs selected for the treatment with candicidin exhibited the lowest base line cholesterol levels prior to treatment, it is interesting to note that the administration of this polyene macrolide at all three dosage levels produced the lowest mean cholesterol levels observed in this study. Those animals treated with amphotericin B and filipin exhibited very similar mean cholesterol levels with relatively small standard deviations. As an exception, filipin administered at the highest dosage level produced the lowest mean cholesterol level for that group.

In grouping the dogs according to the observed base line serum cholesterol levels we are, in a sense, dealing with a distinct population of animals. The groups treated with amphotericin B and filipin were much more hypercholesterolemic than those treated with candicidin and nystatin. Considering the 22 dogs treated with candicidin at the 5 mg/kg level as a distinct group, we found it interesting to plot the percentile of the population versus the serum cholesterol level prior to and after treatment. This is presented in Figure 1. At the 100

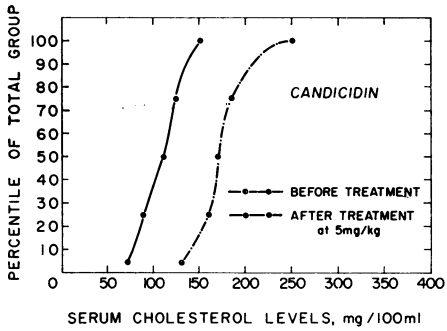

FIG. 1.—Changes in the serum cholesterol levels of the group of dogs prior to and after the oral administration of candicidin at 5 mg/kg.

percentile level all of the untreated dogs exhibited serum cholesterol levels below 250 mg %, whereas after treatment the comparable value was 150 mg %. With this approach to the presentation of the results, it is quite evident that the entire group of dogs exhibited a marked shift in the range of serum cholesterol levels. The posttreatment range of serum cholesterol levels is much lower and narrower. Similar plots are given for amphotericin B and filipin in Figures 2 and 3, respectively. The number of dogs (12) used in these studies with amphotericin B and filipin was limited. However, since there was generally a uniform response at all doses employed, the serum cholesterol levels for these groups of dogs were plotted

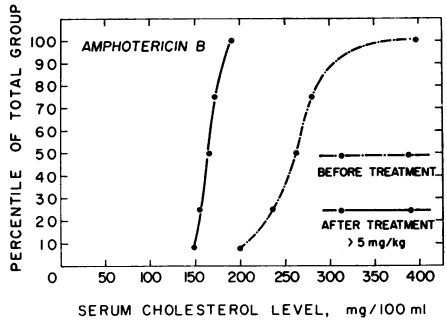

FIG. 2.—Changes in the serum cholesterol levels of the group of dogs prior to and after the oral administration of amphotericin B at 5–40 mg/kg.

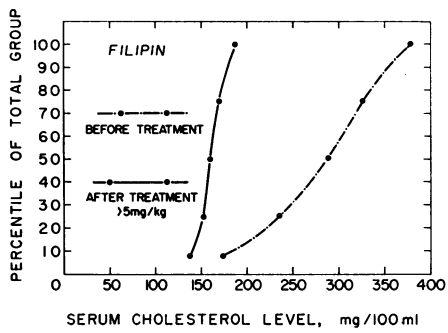

FIG. 3.—Changes in the serum cholesterol levels of the group of dogs prior to and after the oral administration of filipin at 5–40 mg/kg.

together before and after treatment. As was evident with candicidin, the treatment with amphotericin B and filipin at doses of 5 mg/kg and higher produced a significant shift in the range of serum cholesterol levels of the populations. It is clear that the dogs treated with amphotericin B and filipin exhibited prior to treatment generally a higher range of serum cholesterol levels than the group treated with candicidin. Thus, seemingly, the treatment with candicidin at a dosage level of 50 mg/day resulted in a much greater drop in the average serum cholesterol level of the population of 22 dogs. The animals treated with all three polyene macrolides generally revealed a much narrower range in serum cholesterol levels than they did prior to treatment. This suggests a shift to a much greater homeostatic control of the serum cholesterol level after treatment. Those dogs exhibiting the highest serum cholesterol levels prior to treatment exhibited the greatest reduction in cholesterol levels after treatment. Those exhibiting lower levels prior to treatment exhibited much less response to treatment.

Discussion.—The observations made in the dog on the effect of orally administered polyene macrolide antifungal antibiotics on the prostate gland and on serum cholesterol levels may well be related. It is well known that the steroid hormones play a vital role in the development and maintenance of the secondary sexual organs. The relationship of cholesterol to the biosynthesis of steroidal sex hormones is also quite clear; but how the polyene macrolides, upon their oral administration, produce these dramatic physiological effects is as yet unknown.

Considering that the polyene macrolide antifungal antibiotics are generally at best very poorly absorbed from the gastrointestinal tract, we may conjecture that they exert their effect solely within this tract. It is also known that the polyene macrolides generally exhibit very poor solubility in water. When dispersed with water from solutions in polar organic solvents, they produce hydrated colloidal suspensions. As such, it has been established that they interact with certain lipoidal components of cellular membranes of susceptible yeast and fungal cells. This interaction produces a marked alteration of the structure of the membrane, resulting in a disruption of metabolite permeability.

Additional studies currently in progress indicate that the polyene macrolides may possibly interfere with the absorption of exogenous cholesterol. Likewise, the prevention of resorption of endogenous cholesterol in animals with enterohepatic circulation also seems possible. Further evidence on these points is being sought in studies dealing with the fecal excretion of cholesterol and bile acids. The prevention of cholesterol absorption-resorption from the intestinal tract would lower serum cholesterol levels. This would also stimulate cholesterol synthesis in the liver. In the studies with old dogs suffering from benign prostatic glandular hyperplasia, it was evident that the oral treatment with polyene macrolides promoted the utilization of adipose fat. This observation would be consistent with the increased cholesterol synthesis in the liver.

The observations made in the present study and in the prostate hyperplasia studies are certainly related. The effects observed on serum cholesterol levels may have direct bearing on the effects of these compounds on the prostate gland. Cholesterol levels definitely have a direct relationship to steroid hormone levels. The restoration of a much more homeostatic control of cholesterol levels may be the underlying mechanism.

These results provide a simple procedure for producing rather striking and highly reproducible reductions in blood cholesterol levels. The oral administration of the polyene macrolides generally produced the greatest reduction of cholesterol levels when the pretreatment levels were high and the least effect when these levels were low. This establishment of a greatly increased homeostatic control of cholesterol levels is of particular interest.

This development provides great promise as a tool in determining the various aspects of cholesterol metabolism and function. Studies dealing with the effects of these compounds on the course of development of atherosclerosis and other disorders of lipid metabolism should certainly be considered.

Summary.—The oral administration of the polyene macrolide antifungal antibiotics to dogs at dosage levels of 5–40 mg/kg body weight for three weeks resulted in marked reductions of serum cholesterol levels. In review, the heptaene macrolides, candicidin and amphotericin B, and the pentaene, filipin, exhibited great activity; no clear-cut dose response was evident since at all of the dosage levels tested the response was somewhat uniform. After treatment a much greater homeostatic control of serum cholesterol levels was evident. The possible mechanism of action relating to inhibition of cholesterol absorption-resorption from the intestinal tract was discussed.

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