riched with glucose exhibited a pronounced killing effect.

Comment. Spermine is present in rather high concentrations in some human tissues and might play a role as a natural antibacterial substance(8,9). Our findings suggest that spermine might influence *Cryptococcus* infections to a certain degree, but not infections due to *Candida*. *Candida* species were unable to oxidise spermine (unpublished data), hence the high spermine resistance shown by these yeasts cannot be explained by the presence of a spermine oxidase. It is of interest that the highly spermine sensitive *S. cerevisiae* contains appreciable amounts of spermine and spermidine(2). Yeasts adsorbed a smaller amount of spermine than bacteria(7). This phenomenon might be explained by the smaller surface: volume quotient of the yeast cell when compared with that of the bacterial cell. The mechanism of the antimycotic effect of spermine seems to be similar to the antibacterial one. The same factors influence antibacterial and antimycotic action: *i.e.* pH of the medium, presence of inorganic salts, and metabolic activity of the cell.

Summary. 1) Spermine inhibited growth of several species of yeasts, *Saccharomyces* and *Debaryomyces* species being the most sensitive. The antimycotic effect of spermine was more pronounced at an alkaline pH and was antagonized by inorganic salts. The adsorption of spermine by yeast cells was significantly smaller than by *Staph. aureus*. 2) Spermine retarded the death rate of washed cells of *S. cerevisiae* suspended in tris buffer. A pronounced killing effect of spermine was observed when glucose was added to the buffer.

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Effect of Δ-4-Cholestenone on Cholesterol and Phospholipid Metabolism in the Chick. (24384)

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There is increasing interest in the hypothesis that atherosclerosis might be successfully treated by use of materials that inhibit endogenous cholesterol synthesis. One such compound, Δ-4-cholestenone, was first shown by Tompkins *et al.*(1) to diminish the incorporation of labeled acetate into the cholesterol of liver slices taken from rats treated with the material. More recently these investigators (2) reported that *in vivo* cholestenone depressed plasma cholesterol levels in normal dogs and chickens. In addition, Steinberg and Fredrickson(3) demonstrated that when rats were treated with this compound there was a decrease in serum cholesterol and a diminution of incorporation of labeled acetate into hepatic cholesterol. Both groups of workers have emphasized that this compound has serious undesirable side effects, *e.g.*, Chaikoff and colleagues have shown it to be a precursor of cholestan-3β-ol(4) an atherogenic material in both rabbits and chickens(5,6). Furthermore, Steinberg *et al.*(7) have very recently indicated that while cholestenone may have been ineffective in modifying serum cholesterol levels in 2 hypercholesterolemic patients, their

serum dihydrocholesterol concentration was actually augmented by the treatment. These workers(3) demonstrated earlier that a high dose of the compound given to rats produced a profound adrenal hypertrophy and an increase in hepatic total sterol concentration.

Although Δ-4-cholestenone itself may not clinically be suitable as an inhibitor of endogenous cholesterol synthesis, further investigation of its metabolic effects seems warranted since it exhibits a completely different chemical structure and conceivably may block cholesterol synthesis at a different site than another proposed inhibitor of endogenous cholesterol synthesis, α-4-biphenylylbutyric acid (8,9). In the present report we have measured incorporation of acetate-1-C^{14} into the cholesterol, and of inorganic P^{32} into the phospholipid, of plasma, liver, and aorta of chicks treated with Δ-4-cholestenone.

Methods. Exp. 1. Four-week-old cockerels raised on a commercial starter ration were treated for 3 days with 1.5% Δ-4-cholestenone added to the diet. Control animals received the starter mash alone. The animals were fasted overnight, then given intravenously 20 microcuries (1.48 mg) of sodium acetate-1-C^{14} and 200 microcuries (1.45 mg) of P^{32} labeled sodium phosphate. Thirty, 60, and 240 minutes after administration of the isotopes, plasma samples and liver biopsies were taken, and at the 4 hour interval the animals were sacrificed and the thoracic aortae removed. Plasma and homogenized tissue samples were extracted with chloroform:methanol (2:1) and washed overnight in running water as a modification of the method of Folch *et al.*(10). The solvents of aliquots of the chloroform:methanol extracts were removed by gentle heat and the lipids saponified with 30% alcoholic KOH. The non-saponifiable fraction was extracted with petroleum ether and the cholesterol in this extract was determined by the method of Zlatkis *et al.*(11). Aliquots of the lipid extracts were mounted on lens paper discs supported by copper planchets and radioactivity was measured by an end-window geiger tube. There was no contamination of the petroleum ether extracts by P^{32}. Although other sterols in the petroleum ether extracts may have been labeled with C^{14} we have assumed the radioactivity of these extracts was due primarily to labeled cholesterol. Phospholipid phosphorus was estimated in aliquots of the washed chloroform:methanol extracts by the method of King(12). P^{32} concentration in the extracts was determined on aliquots mounted as described above. C^{14} radiation from these samples was absorbed with aluminum. In calculating the specific activities of all lipid extracts corrections for background, radioactive decay, and absorption have been included. *Exp. 2.* Five-week-old cockerels were treated with 0.1% Δ-4-cholestenone in the diet for the period of 12 days. Control animals received the starter mash alone. After an overnight fast all birds were given 20 microcuries of acetate-1-C^{14}. Plasma and tissue sampling and analysis were carried out as described above.

Results. Cholesterol and phospholipid concentrations. Plasma and tissue cholesterol and phospholipid concentrations in both experiments are exhibited in Table I. Treatment with cholestenone produced a slight but

TABLE I. Cholesterol and Phospholipid Concentrations in Plasma, Liver, and Aorta in Chicks Treated with △-4-Cholestenone.

		Exp. 1				Exp. 2	
	Time,	Cholesterol (mg/g)*		Phospholipid (mg/g)*		Cholesterol (mg/g)*	
Tissue	min.	Control	Treated	Control	Treated	Control	Treated
Plasma	30	1.01 ±.16	.94 ±.07	1.86 ±.24	1.06 ±.02†	.743 ±.020	.668 ±.033
	60	.79 ±.15	.94 ±.05	1.66 ±.13	.98 ±.08†	.671 ±.018	.606 ±.027
	240	.94 ±.17	.81 ±.09	1.13 ±.13	.83 ±.09	.642 ±.058	.543 ±.022†
Liver	30	5.54 ±.69	4.19 ±.45	21.9 ±.02	23.8 ±.14	3.50 ±.001	3.51 ±.09
	60	5.93 ±.99	5.45 ±.36	20.8 ±.10	25.2 ±.30†	3.32 ±.14	3.31 ±.06
	240	5.20 ±.79	4.07 ±.36	25.0 ±.06	26.1 ±.03	3.12 ±.03	3.04 ±.10
Aorta	240	3.61 ±.45	2.48 ±.21‡	8.79 ±.04	8.19 ±.27	2.21 ±.09	1.77 ±.14 †

* Mean and stand. error of 3 or more animals. † $P < .01$; ‡ $P < .05$; unmarked treated values are not significantly different from those of the controls.

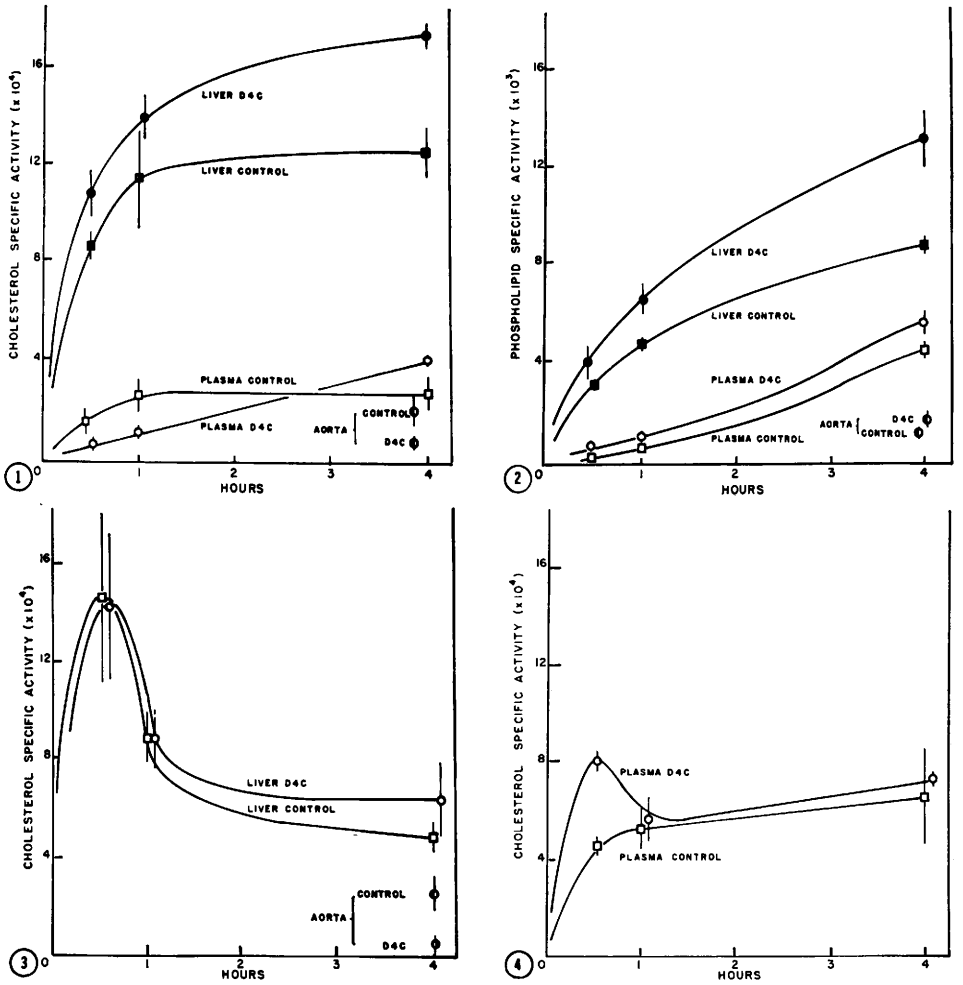

FIG. 1. Cholesterol specific activity-time curves for Exp. 1. Ordinate shows cholesterol specific activity expressed as % of the administered C^{14} recovered in the non-saponifiable fraction per mg of cholesterol. Each point is mean of 3 or more animals; vertical lines indicate standard errors. Symbol D-4-C has been used as an abbreviation for Δ-4-cholestenone.

FIG. 2. Phospholipid specific activity-time curves for Exp. 1. The ordinate indicates the specific activity of the phospholipid expressed as % of administered P^{32} recovered per mg of phospholipid. Each point is the mean of 3 or more animals; vertical lines indicate standard errors. The symbol D-4-C has been used as an abbreviation for Δ-4-cholestenone.

FIG. 3. Cholesterol specific activity-time curves for liver and aorta in Exp. 2. Ordinate and symbol are as in Fig. 1.

FIG. 4. Cholesterol specific activity-time curves for the plasma in Exp. 2. Ordinate and symbol are as in Fig. 1.

statistically insignificant decrease in plasma cholesterol levels in both tests. Liver cholesterol concentrations were not affected by the treatment. The aorta showed a significant diminution in cholesterol content as a result of either acute or sub-acute administration of the compound. The phospholipids of the plasma were significantly reduced in the treated animals but neither liver nor aorta showed significant changes in concentration of this lipid.

Cholesterol and phospholipid specific activities. Specific activity-time curves for plasma, liver, and aorta cholesterol in Exp. 1 are shown in Fig. 1. Treatment with cholestenone noticeably enhanced incorporation of acetate in hepatic cholesterol. Although this effect seemed not to be obvious at the earlier time intervals, 240 minutes after isotope ad-

ministration, plasma cholesterol specific activity rose above that of the control to a significant amount. As in the case of cholesterol concentration, cholesterol specific activity of the aorta was profoundly depressed by the cholestenone treatment. Fig. 2 exhibits specific activity-time curves for tissue phospholipids. Liver and plasma showed significant enhancement of incorporation of P^{32} into the phospholipid as a result of cholestenone treatment, while the effect upon the aorta was minimal.

In Exp. 2 the differences between control and treated groups have been minimized by the change in method of treatment (Figs. 3 and 4). As in the first trial, however, there is inhibition of the incorporation of acetate into cholesterol in the aorta of the treated group.

When the plasma and liver cholesterol specific activity-time curves for the 2 experiments are compared, noticeable differences in shape are evident. Inasmuch as both the treated group and its control roughly parallel one another in each experiment, these differences must be ascribed to the variability of response between the 2 lots of birds.

Discussion. The data presented here do not completely confirm those reported by Chaikoff's group for the chicken nor those for the rat given by Steinberg and Fredrickson. The differences however seem in the main quantitative and are probably due to differences in experimental design. The California workers(2) reporting on the treatment of 2 chickens with Δ-4-cholestenone, described a 50% drop in plasma cholesterol levels after a 10- to 16-day period of feeding a diet containing a 1% of the sterol. In the present report we have observed only 10 to 15% decrease in plasma cholesterol levels as a result of addition of 0.1% cholestenone to the diet for 12 days or of 1.5% cholestenone to the ration for only 3 days.

Steinberg and Fredrickson(3) reported that in the rat chronic treatment with a high dose (1% in the diet) of cholestenone resulted in a decrease both in the plasma cholesterol level and in incorporation of acetate-1-C^{14} into hepatic non-saponifiable lipid.

Our demonstration in this report that acute treatment of chicks with Δ-4-cholestenone may actually enhance incorporation of acetate-1-C^{14} into cholesterol emphasizes an apparent species difference between the young bird and the rat with respect to their responses to cholestenone treatment.

The enhancement of both phospholipid and cholesterol metabolism in the liver suggests that a general increase in cellular activity in this organ may have resulted from the cholestenone treatment. Following up this concept we have tested the hypothesis that Δ-4-cholestenone may have choleretic activity, and a preliminary investigation has shown that in chicks fed 1.5% Δ-4-cholestenone for 3 days the fasting biliary cholesterol excretion might be 4 times the control value. If these preliminary studies on bile flow can be confirmed, then it is evident that Δ-4-cholestenone may have a dual action upon hepatic cholesterol metabolism in the bird. At low, non-choleretic doses the material conceivably may inhibit cholesterol synthesis in the liver, while at higher doses cholestenone stimulation of bile flow may bring about an augmentation of cholesterol synthesis that overrides the inhibiting effects of the drug. The observed changes in hepatic cholesterol metabolism would be the resultant of these opposing factors.

Consideration of the data on the aorta again emphasizes that the lipid metabolism of this tissue is unique(9) when compared with the liver. Straight-forward inhibition of synthesis of cholesterol leading to a decrease in the aortic cholesterol content is the obvious interpretation of these data. No effect was apparent upon aortic phospholipid metabolism. One of the most interesting observations was the fact that this compound actually decreased aortic cholesterol concentration.* Apparently degradation or mobilization of this sterol continued, unaffected by a significant decrease in cholesterol synthesis. If this phenomenon can be shown to obtain in the mammal, then it may have important therapeutic implications.

Summary. Incorporation of acetate-1-C^{14}

* It is, of course, conceivable that aortic cholesterol has been passively replaced by some other sterol not detected by our analytical methods. A study testing this hypothesis is in progress.

and P^{32} labeled phosphate into the cholesterol and phospholipids of plasma, liver, and aorta of chicks treated with Δ-4-cholestenone was measured. The response of the chick differed from that of the rat in that there was an enhancement of the inclusion of the radioactive labels into their respective products in plasma and liver. A significant decrease in aortic cholesterol content and specific activity was noted.

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Infection of Mice with G Variants (Micro-Colonies) of *Brucella abortus*. (24385)

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Hall and Spink(1) isolated a streptomycin-resistant strain of *Brucella abortus* in 1946 from blood of human patient with bacterial endocarditis due to *Br. abortus*, who had been treated with streptomycin. On agar plates this strain was composed mostly of minute colonies of brucellae having biochemical and serological characteristics of *Br. abortus*. These small colonies were markedly streptomycin-resistant, yet sublethal concentrations of the antibiotic stimulated growth, in marked contrast to the highly streptomycin-susceptible culture that had been obtained from blood of patient just prior to therapy with streptomycin. This small-colony strain was maintained in the laboratory for 7 years through many subcultures. Subsequently, Huddleson and Baltzer(2) in a study of the dissociation pattern of brucella isolated what they termed a "Type G (Micro-Colony Type) of *Brucella abortus*" from 10 of 12 strains of CO_2 dependent *Br. abortus*. They successfully infected guinea pigs with these micro-colonies, and postulated that these small forms of brucella might very well have been overlooked previously in material cultured from tissues and body fluids of infected animals and man. They emphasized that these micro-colonies were not the same as those described by Pickett and Nelson(3). Further studies in our laboratory were also concerned with the G forms of *Micrococcus pyogenes var. pyogenes*. Wise and Spink(4) reviewed the available information on micro-colonies originating from different species of bacteria, and pointed out that small-colony forms of staphylococcus could be readily selected out *in vitro* by several antibiotics. Evidence suggested this phenomenon also occurred in human patients who had been treated with antibiotics. Infections in animals were successfully established with G forms of staphylococci. The present report concerns investigations on brucella micro-colonies obtained *in vitro* from smooth strains of *Br. abortus* previously isolated from human patients. Growth characteristics, biochemical and serological properties, and *in vitro* susceptibility of these forms to the ac-