Conclusions. It is evident that use of SC-12937 as a hypocholesterolemic agent induced the appearance of desmosterol in sera of rats and man. In addition, in man traces of 2 other sterols were detected. In this regard it is noteworthy that Klein *et al*(7) have found that in the rat subjected to short interval, low dose treatment with SC-12937 the appearance of an unidentified sterol was detected *without* desmosterol accumulation. Further, Thompson *et al*(8) detected in the carcasses of rats treated with a high dose (10 mg/kg for 8 weeks) of SC-12937 a sterol they tentatively identified as 7,24-cholestadiene-3β-ol. These investigators found only cholesterol and desmosterol present in the livers, however. It seems evident that the discrimination between cholesterol and the other sterols that might appear in the serum or tissues as a result of SC-12937 treatment may be more accurately carried out by gas chromatographic analysis or high resolution column chromatography(9) of the nonsaponifiable lipid than by spectrophotometry.

The hypocholesterolemic effect of SC-12937 may be tentatively attributed in large part to inhibition of the synthesis of cholesterol at multiple loci in the series of poorly understood transformations whereby lanosterol is converted to cholesterol. The particular cholesterol precursor(s) that may be detected during SC-12937 treatment seemingly will be dependent upon the intensity and duration of the treatment with the drug.

Summary. Analysis of the plasma of rats and patients treated with 20,25-diazacholesterol dihydrochloride demonstrated the presence of desmosterol in the non-saponifiable lipids. Small amounts of 2 unidentified sterols were also detected in the human plasma samples.

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Treatment of Experimental Salmonellosis in Chicks with Synnematin B* (29431)

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Following the observation that synnematin B† exerted an *in vitro* bacteriostatic effect on *Salmonella*(1), Olson and Jennings found that in mice or chicks the visceral organs of animals inoculated with *S. typhimurium*, *S. typhi* or *S. pullorum* could be freed of these microorganisms even if treatment with the antibiotic was delayed 1-2 days or more(2). Others confirmed the life-saving effect of synnematin B in mice, when drug administration was begun within an hour after exposure to *S. typhimurium*(3,4), although Hobby *et al* observed that the organisms localized in the spleens could not be completely eradicated

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† Syn.: adcillin, cephalosporin N, penicillin N.

during 14 days of treatment. Based partly on the foregoing as well as on surveys which showed a high degree of *in vitro* susceptibility among many strains of *Salmonella*(5,6), preliminary therapeutic trials with synnematin B were carried out in infected humans. The clinical response to treatment was considered generally good, particularly in typhoid fever(7,8). In illnesses caused by other species, sterilization of infection was not regularly achieved and it was concluded that further experience would be required to assess the potentialities of this drug *vs* human salmonellosis(9).

Earlier work in our laboratory had shown that newly hatched chicks were highly susceptible to peroral infection with small numbers of *Salmonella* of many varieties, providing a useful model for studying some features of pathogenesis and chemotherapy(10, 11). In such a system several other antibiotics, including chloramphenicol, had failed to achieve more than a temporary suppression of excretion of the microorganisms; thiocymetin (Win 5063-2), which differs from chloramphenicol by the substitution of a methyl sulfonyl group for the nitro group, had been shown in some instances capable of sterilizing infections if given in doses approaching a toxic level for the birds(12,13). It therefore seemed of interest to compare the performance of synnematin B.

Materials and methods. The *Salmonella* used had been tested previously for ability to infect chicks perorally. New Hampshire Red eggs (Christie Farms strain) were incubated in our laboratory and the chicks inoculated within 48 hours of hatching. They were fed on an antibiotic-free mash. Methods for preparing and administering bacterial inocula, handling infected animals and making bacteriological examinations of cloacal swabs or visceral tissues have been described(10, 11), as well as the response of such animals to treatment with certain antimicrobial drugs (12,13). In virtually every animal, infection with the strain inoculated was demonstrated by cloacal swab culture taken just prior to initiating chemotherapy; consequently, detailed data on the pre-treatment cultures are omitted below.

Synnematin B was kindly provided by the Division of Laboratories, Michigan Dept. of Health, or by the Abbott Laboratories. Aqueous solutions of drug were used for injection or bioassay. Minimal inhibitory concentrations (MIC) for various *Salmonella,* or serum levels, were measured by a method utilizing doubling dilutions in tubes. For parenteral administration the antibiotic was given subcutaneously, using alternate legs for successive injections to avoid disabling trauma and to obtain relatively good absorption from the loose connective tissue in this area. Peroral doses were given in gelatin capsules.

Results. The MIC of synnematin B *in vitro vs* the various strains used for infecting chicks was found in each instance to be 0.8-1.6 units/ml (with inoculum of $\pm$ 10,000 viables), comparable to values obtained by other workers(1,4,6,9). When attempts were made to measure serum levels following a single subcutaneous dose, the MIC was not attained with 2000 units; with 4000 units a level at or slightly above 1.6 units/ml could be detected for periods up to 4 hours but not 6 hours. No activity was found in the serum of chicks given 4000 units perorally; absorption of the drug from the alimentary tract was evidently poor in the birds, as in other animals and humans(14,15). Subcutaneous injection of doses as large as 8000 units at intervals of 8 hours over a period of 7 days did not produce any significant local lesions or signs of systemic discomfort; the chicks ate well and gained weight at a rate comparable to that of untreated controls.

Since *S. typhimurium* had been employed by previous workers to show *in vivo* activity of synnematin B, we carried out experiments with strain 5609 which had been found useful in evaluation of other drugs(12,13). Table I summarizes the pertinent data for 5 trials, in which the dosage schedule and period of treatment were varied. Subcutaneous doses of 4000 units appeared to effect a significant temporary reduction in the proportion of animals shedding *Salmonella* in the cloacal contents during the period of treatment. Even after 2 days or more following drug discontinuance, some animals did not resume excretion of the microorganisms.

TABLE I. Cultural Findings in Chicks Infected Perorally with *Salmonella* and Subsequently Treated with Synnematin B.

Salmonella Tested			Exp.	Pre-treatment Interval (Days)	Treatment			Number Positive/Number Examined				All Terminal Cultures Negative*
								Cloacal Cultures After		Visceral Cultures† (Terminal)		
Type	Strain	Inoculum (Viables)			Route	Interval Between Doses (Hours)	Duration of Treatment (Days)	3-7d. of Treatment	2-5d. Post-Treatment	Animals	Specimens	
typhimurium	5609	290	250	1	S.C.° None	8 -	7 -	1/7 7/7	5/7 6/6	4/7 7/7	9/35 21/35	2/7 0/7
		470-960	247 + 252	1	S.C. S.C. + P.O. None	8 8 -	7 7 -	0/8 1/15 24/24	2/8 1/14 19/19	1/8 1/15 24/24	2/40 1/75 77/120	6/8 13/15 0/24
		1940	257	1	S.C. None	4 -	5 -	1/8 6/6	5/8 4/4	5/8 8/8	9/40 35/40	2/8 0/8
		2100	258	2	S.C. + P.O. None	4 -	5 -	0/8 6/6	0/8 4/6	3/8 8/8	3/40 33/40	5/8 0/8
	B-101	440	250	1	S.C. None	8 -	7 -	2/7 6/6	6/7 3/3	6/7 7/7	11/35 29/35	1/7 0/7
		4500	256	3	S.C. + P.O. None	8 -	7 -	0/8 5/5	2/5 5/5	1/8 7/8	1/40 24/40	6/8 0/8
paratyphi B	275 E	2300	257	1	S.C. None	4 -	5 -	1/8 6/6	6/8 4/4	2/8 8/8	2/39 32/39	1/8 0/8
		2300	258	2	S.C. + P.O. None	4 -	5 -	1/8 7/7	3/8 6/6	2/8 8/8	4/40 33/40	5/8 0/8
montevideo	B-33	550	248	1	S.C. None	8 -	7 -	6/8 8/8	8/8 8/8	5/8 7/8	10/40 14/40	0/8 0/8
		180	253	1	S.C. + P.O. None	8 -	7 -	1/7 8/8	6/7 7/8	3/7 7/8	6/35 14/40	1/7 0/8
		5200	256	3	S.C. + P.O. None	8 -	7 -	6/8 8/8	8/8 8/8	2/8 8/8	4/40 29/40	0/8 0/8
		1500	257	1	S.C. + P.O. None	4 -	5 -	0/7 8/8	2/7 7/7	1/8 8/8	1/40 16/40	5/8 0/8
		1400	258	2	S.C. + P.O. None	4 -	5 -	2/8 8/8	8/8 8/8	2/8 8/8	4/40 21/40	0/8 0/8
enteritidis	CAE	320	248	1	S.C. None	8 -	7 -	2/8 8/8	5/8 6/8	4/8 6/8	7/40 9/40	3/8 0/8
		260	253	1	S.C. + P.O. None	8 -	7 -	1/7 8/8	6/7 8/8	3/7 7/8	6/35 14/40	1/8 0/8
		1300	257	1	S.C. None	4 -	5 -	1/8 8/8	5/8 6/7	3/8 8/8	7/40 23/40	3/8 0/8
		3500	258	2	S.C. + P.O. None	4 -	5 -	2/8 7/8	5/8 5/8	1/8 8/8	1/40 24/40	3/8 0/8
meleagridis	5644	680	250	1	S.C. None	8 -	7 -	3/6 6/6	5/6 5/6	3/6 4/6	5/30 5/30	1/6 0/6
		300	253	1	S.C. + P.O. None	8 -	7 -	2/7 8/8	6/7 8/8	5/7 2/8	7/35 5/40	0/7 0/8

° S.C. = subcutaneous; P.O. = peroral (each dose by either route, 4000 units).

† Figures in these columns relate to all animals in a group, some of which may have died prior to termination of experiment.

* No *Salmonella* recovered from cloacal swabs or any of 5 visceral cultures.

Compared with untreated controls, significantly fewer animals in the treated groups yielded *Salmonella* from one or another of the viscera (heart, lung, liver, spleen, gall bladder) which were routinely cultured when animals were found dead or were sacrificed on termination of experiments 2-5 days following cessation of chemotherapy. The groups of treated birds also showed a smaller total number of such visceral sites harboring viable *Salmonella*. In each trial some treated birds yielded no organisms from either the cloaca or any visceral site, implying sterilization of the infection. Combination of equal peroral doses with those given subcutaneously may have improved results slightly, but the findings were similar whether drug was given for 5 days at intervals of 4 hours or for 7 days at intervals of 8 hours.

In Table I are also presented findings from trials with 5 other strains of *Salmonella* representing Kauffmann-White groups B-E. Infections with *S. typhimurium* B-101 responded to synnematin B like those due to

strain 5609, with combined subcutaneous and peroral dosing producing somewhat more effective elimination from the gut and viscera even though initiation of treatment was delayed by 2 additional days. The trend of results was similar with *S. paratyphi B, S. montevideo* and *S. enteritidis*. Only with *S. meleagridis* did there appear to be no appreciable benefit from synnematin B.

Discussion. In the doses employed here, 4000 units injected subcutaneously in chicks initially weighing 30-60 g, the antibiotic was well tolerated but bacteriostatic serum levels were maintained for not more than 4 hours so that in animals treated at 8-hourly intervals there were undoubtedly periods when subinhibitory concentrations were present in the blood. From studies in other animal species, synnematin B is known to be excreted largely *via* the urine(2,14,15). The chicken cloaca contains a mixture of urinary and fecal material, so this might perhaps account for negative cloacal cultures obtained during the period of chemotherapy. However, failure to recover *Salmonella* from swabbings 2 days or more after discontinuing treatment would indicate an effect of the parenterally administered drug on excretion of microorganisms *via* the gut; such effects have been noted in typhoid patients treated with synnematin B(16). When similar doses were given by gavage as well as subcutaneously, further beneficial effect was sometimes observed. It may be pointed out that in salmonellosis patients treated by de la Torre and Olarte(9), when combined peroral and intramuscular routes were used complete bacteriological cures were likewise seen. Even though the dosage schedules used did not provide high serum levels continuously, antibiotic reaching the visceral tissues seemed to have an appreciable bactericidal effect within the 5-7 days of treatment, bearing out the *in vitro* observations of Jennings and Olson (17) and those made by Hopps *et al* using tissue cultures(18), as well as clinical studies(19,20). In this regard synnematin B would have an advantage over other drugs which have been employed against *Salmonella* but which do not bring about death of the microorganisms. In our tests the range of action of synnematin B *in vivo* extended to several *Salmonella* species but was not universal, as shown by the lack of significant effect on infections by *S. meleagridis* 5644 even though *in vitro* the latter was not more resistant than the other strains tested. Such variability of results has been observed in human infections also(9). Nevertheless, further exploration of the clinical usefulness of the antibiotic would seem to be warranted.

Summary. In chicks treated with synnematin B by subcutaneous injection beginning 1-3 days after peroral infection by various *Salmonella* species, there was evidence of a bactericidal effect against microorganisms localizing in visceral organs and, to a limited extent, those in the alimentary tract. Although the antibiotic was not appreciably absorbed from the gut, combined peroral and subcutaneous treatment was sometimes advantageous. Some infections did not yield to chemotherapy even though *in vitro* tests indicated the responsible *Salmonella* strains to have a considerable degree of susceptibility to synnematin B.

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Studies on 2-Deoxy-D-Glucose-Induced Hyperglycemia.* (29432)

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It has been demonstrated that 2-deoxy-D-glucose (2-DG) is a powerful inhibitor of glucose utilization *in vivo*(1-5). This inhibition brings about hyperglycemia, which is increased further by the release of epinephrine from the adrenal medulla(6). Other studies have shown that the cellular glucopenia induced by 2-DG produces certain histologic changes in the pancreatic islets, which suggest increased activity of both the A- and the B-cells(7). The purpose of this study was to find out whether a stimulation of pancreatic A-cells and an increase in glucagon secretion contribute to 2-DG-induced hyperglycemia.

Materials and methods. Mongrel dogs of both sexes, weighing between 10 and 15 kg were fasted overnight and anesthetized with pentobarbital sodium (35 mg/kg I.V.). The pancreato-duodenal (P-D) artery was cannulated as described previously(8). 2-DG (500 mg, standard dose per animal, dissolved in saline 1 ml/kg) was injected into the artery, a technique which allows perfusion of the pancreas with a relatively high concentration of 2-DG, while leaving the rest of the organism unaffected by the sugar. Control injections were made into an exposed femoral vein. Some animals were pretreated with dihydroergotamine (DHE; 0.4 mg/kg), injected intravenously 35 minutes before injection of 2-DG.

Blood samples were obtained from a femoral vein for duplicate glucose determinations according to Nelson(9). The statistical significance of the results was calculated according to Fisher(10).

Results. All results are given in the form of curves representing changes in blood sugar concentration from the average of 3 pre-injection values. These were taken 30 minutes, 15 minutes and immediately before the injection of 2-DG.

The results show that injections of 2-DG into the P-D artery caused a statistically significant hyperglycemia ($p<0.01$) lasting approximately 30 minutes. A still greater hyperglycemic effect, lasting approximately 45 minutes, was obtained by injecting 2-DG into the femoral vein ($P<0.001$). No changes from pre-injection levels were observed in animals pretreated with DHE. Previous experiments had demonstrated that injections of saline into the P-D artery do not modify blood glucose concentration significantly(8).

Discussion. These studies confirm the hyperglycemic effect of 2-DG, even in doses 10 times smaller than those used by other investigators. They also confirm the fact that DHE blocks this hyperglycemic response. The role of the pancreas in these phenomena is not clear. The fact that the hyperglycemic

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