protamine in the presence of added RNA, DNA or PES. Control homogenate preincubated without protamine maintained a level of ATP while homogenate preincubated with protamine was devoid of ATP. Homogenate preincubated with protamine plus PES maintained a level of ATP equal to the control. Protamine inhibition also could be reversed with large amounts of ATP. Evidence that protamine combines with factors, presumably "free" or "combined" RNA, concerned with maintenance of ATP levels rather than with ATP *per se*, was found by activity of ATP in an *in vitro* enzyme system that requires ATP was not influenced by protamine to ATP levels comparable to those involved in the biosynthetic experiments. Our findings lead to the interesting question, implied or discussed by a number of authors, of the extent to which control of biochemical activity within the cell is regulated *via* basic proteins. It appears that if a strongly basic protein such as protamine were elaborated intracellularly activity requiring ATP might be depressed as a consequence of unavailability of "free" or "combined" RNA. On the other hand, some degree of RNA-protein combination appears essential to protect RNA from intracellular RNAse. Our studies have shown that "nucleoprotein" of autoclaved liver extract is resistant to RNAse but after separation of the moieties the RNA is then susceptible to RNAse. Thus a number of factors that have been loosely characterized in the literature dealing with "tightness" with which phosphorylation is coupled (or uncoupled) to oxidation may represent proteins of varying affinity for polyanions of the cell that seem to be concerned with maintenance of ATP levels.

Summary. Protamine is a potent inhibitor of a system essential for conversion of mevalonic acid to cholesterol. Evidence was presented that protamine combines with a polyanion, presumably "free" or "combined" RNA, that is essential for maintenance of an ATP level required in utilization of mevalonic acid.

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Effect of Neomycin on Cholesterol Atherosclerosis in the Rabbit. (25696)

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A significant decrease in serum cholesterol has been recently observed in 10 patients following oral administration of the antibiotic Mycifradin which contains 70% neomycin sulfate(1). However, Broitman *et al.*(2) observed a marked hypercholesterolemia and valvular sudanophilia in neomycin-treated rats fed a cholesterol and cholate diet. In both studies the effect of neomycin was attributed to alterations of intestinal bacterial flora or certain enzyme systems of the gastrointestinal tract. The purpose of this report is to note the effect of oral and parenteral administration of this agent on serum lipids of normal and cholesterol-fed rabbits and the

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TABLE I. Effect of Neomycin on Body Weight, Coliform Count of Stool and Aortic Atherosclerosis.

Group	Diff. wt (kg)	Coliform count/g		Degree of As*
		Beginning	End	
I Oral neomycin + chol.	+ .6	6.2×10^8	4.1×10^2†	1.75
II Chol.	+ .5	4.3×10^7	6.6×10^8	.75
III Intramusc. neomycin + chol.	+ .7	5.2×10^7	3.6×10^7	.83
IV Oral neomycin	+1.0	7.3×10^8	6.0×10^3†	0
V Intramusc. neomycin	+1.1	6.2×10^7	4.7×10^6	0

* Avg of thoracic and abdominal segments (maximum 3.0). † $P < .01$.

atherosclerotic process associated with the latter.

Material and method. Forty adult, white rabbits initially weighing 2 kg were divided into 5 groups, each containing equal numbers of both sexes. Group I consisted of 12 animals maintained on 2% cholesterol diet and drinking water containing 0.5% (w/v) neomycin† Eight rabbits of Group II maintained on cholesterol diet and drinking water without neomycin. Group III comprised 8 animals receiving cholesterol diet and daily intramuscular injections of 50 mg of neomycin. Group IV consisted of 6 animals maintained on stock laboratory diet without cholesterol supplement and drinking water containing 0.5% neomycin. Group V consisted of 6 animals maintained on stock cholesterol-free diet and tap water but received daily intramuscular injections of 50 mg of neomycin. All animals were sacrificed after 70-72 days of treatment. Animals receiving neomycin orally consumed a total of 30-40 g of the drug during experimental period. Blood was obtained by cardiac puncture prior to sacrifice and total and esterified cholesterol determined by Schoenheimer and Sperry method(3), total lipids according to de la Huerga *et al.*(4), and lipid phosphorus by the method of Youngburg and Youngburg(5). Coliform counts were made on samples of stool before initiation of experiments and at time of sacrifice. Degree of aortic atherosclerosis was estimated at necropsy as described previously(6). Organs and aorta were routinely stained with hematoxylin and eosin after fixation of tissue in 10% neutral formalin. Blood pressure was determined on ½ of animals of each group by the ear-capsule technic of Grant and Rothschild(7).

Results. No toxic effects of neomycin were noted. All animals receiving cholesterol diet exhibited less weight gain than rabbits maintained on cholesterol-free, stock laboratory diet although food consumption was considered comparable (Table I). Prolonged administration of neomycin orally or parenterally failed to influence these changes in body weight. Coliform counts on aliquots of stool were significantly reduced only in animals receiving neomycin orally whether maintained on cholesterol or stock laboratory diets. Elevation of serum lipids (Table II) attendant with cholesterol feeding was significantly greater in rabbits subjected to oral neomycin (Group I) than in those of Group II that did not receive this drug. Similarly, aortic atherosclerosis as indicated in Table I and Fig. 1 and visceral lipoidosis were greater in the former (Group I). Serum lipids and degree of atherosclerosis were similar in cholesterol-fed rabbits subjected to intramuscular injections of neomycin (Group III) and those not receiving this drug (Group II). Administration of neomycin to animals not receiving cholesterol failed to alter their serum lipids or normal appearance of the aorta and viscera. All animals remained normotensive and differences observed were not related to sex.

Histologic study failed to disclose significant qualitative differences in atherosclerotic lesions encountered in neomycin-treated or untreated animals fed cholesterol. No evidence of renal damage was apparent in animals receiving neomycin.

Comment. The effect of orally administered neomycin in cholesterol-fed rabbits appears similar to that observed in the rat by Broitman *et al.*(2), producing a greater eleva-

† Administered as Mycifradin Sulfate; generously furnished by Upjohn Co.

TABLE II. Effect of Neomycin on Serum Lipids.

Group	No.	Cholesterol (mg %) Total	Cholesterol (mg %) Esters	Total lipid (mg %)	Lipid P (mg %)
I Oral neomycin + chol.	12	1580 ± 229	666 ± 143	2275 ± 250	32 ± 6
II Chol.	8	938 ± 264	373 ± 140	1573 ± 254	20 ± 8
III Intramusc. neomycin + chol.	8	963 ± 218	390 ± 80	1370 ± 230	19 ± 7
IV Oral neomycin	6	110 ± 10	68 ± 10	247 ± 63	3 ± .05
V Intramusc. neomycin	6	101 ± 6	61 ± 6	210 ± 71	3 ± .06

P groups I & II, I & III <.01. P groups II & III, IV & V >.1.

tion of serum lipids and a more severe degree of aortic atherosclerosis than in untreated, cholesterol-fed controls. Accentuation of visceral lipoidosis was also observed in orally treated, cholesterol-fed rabbits. Although oral administration of neomycin resulted in marked elevation of serum lipids in the cholesterol-fed rabbit this agent was without effect on endogenous cholesterol metabolism in this species. These findings are unlike those observed in man in whom significant decreases of serum lipids have been noted following oral administration of neomycin(1). The greater degree of aortic atherosclerosis and visceral lipoidosis resulting from oral administration of neomycin to cholesterol-fed rabbits appears related to the greater degree of hypercholesterolemia in these animals. Other factors noted to influence cholesterol atherosclerosis in the rabbit such as undernutrition, hypertension or alteration of the cholesterol:phospholipid ratio were not apparent. Although the exact mechanism responsible for the effects of neomycin in the rabbit is inapparent it appears reasonable to assume that it is related to increase in intestinal absorption of cholesterol. Intramuscular injections of neomycin failed to alter degrees of lipemia or aortic atherosclerosis in treated cholesterol-fed rabbits from that observed in cholesterol-fed controls. It appears significant that orally administered neomycin is highly stable in the intestinal tract and is almost totally (97%) eliminated by this route, whereas that following intramuscular injection is excreted in the urine. Whether the accentuating effect of orally administered neomycin on cholesterol atherosclerosis is related to depression of intestinal bacterial flora resulting from administration of this drug remains to be demonstrated.

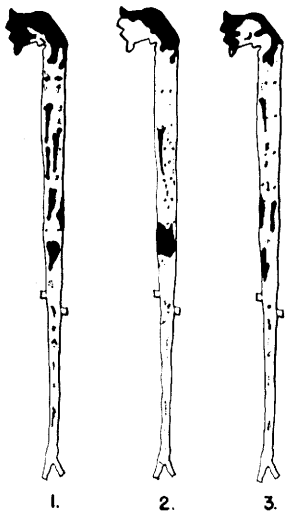

FIG. 1. Schematic representation of degree and distribution of aortic atherosclerosis. 1. oral neomycin, cholesterol-fed; 2. untreated, cholesterol-fed; 3. intramusc. neomycin, cholesterol-fed.

Summary. Prolonged oral administration of neomycin resulted in a greater elevation of serum lipids and more severe aortic atherosclerosis and visceral lipoidosis in cholesterol-fed rabbits than in untreated cholesterol-fed controls. Failure to observe these enhancing effects following intramuscular administration of neomycin indicates that this action is related to increased absorption of cholesterol. Neomycin was without effect on endogenous cholesterol metabolism in this species.

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Peripheral Metabolism of Thyroxine and Triiodothyronine in Iodine Deficient Rat.*† (25697)

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In the iodine deficient rat, iodide trapping activity and rate of hormonal secretion by thyroid are markedly enhanced(1). Morphologic and metabolic aspects of the gland indicate extreme activity while there is insufficient output of hormones. The present experiments were therefore undertaken to determine whether the iodine deficient state influences peripheral disposition of thyroidal hormones, more particularly, rate of disappearance from serum of radiothyroxin (T4*) and radiotriiodothyronine (T3*), their space of distribution and quantity of hormone degraded daily.

Materials and methods. In each experiment, 14 male rats were divided into 2 groups of 7 each. One group was placed on iodine deficient diet‡ and distilled water for 5 weeks; the control group received tap water and pellet diet containing 43 μg of KI/100 g of diet. Then, all rats were injected subcutaneously with 50 mg of propylthiouracil (PTU) in 0.5 ml of saline and 15 minutes later *via* tail vein with about 20 μc of radiohormone.§. They were bled from tail vein at 6 and 12 hours and every 12 hours thereafter until autopsy, at which time they were exsanguinated. For T4*, period of observation lasted 72 hours, and for T3* 48 hours. Urines and feces were collected for each 24 hour period. At autopsy, thyroids were removed, cleaned of adventitial tissue, and weighed on torsion balance to the nearest 0.1 mg. Radioactivity in thyroids, urine, feces and whole carcass (minus blood and thyroids) was counted. A measured aliquot of serum from each bleeding was counted for total and Somogyi precipitable radioactivity. Half-lives of radiohormones were determined by inspection of the disappearance curves, constructed on semi-log paper plotting serum radioactivity against time in hours. Space of distribution was calculated from total radioactivity injected, divided by counts/min/ml of serum at zero time, the latter figure obtained by extrapolating the straight line of disappearance curve to the ordinate axis. Chromatograms were made from extracts of each bleeding and from each 24 hour urine collection. One ml of serum from each animal of a group was pooled for each bleeding. Pooled sera and a mixture of mono-iodotyrosine, diiodotyrosine, triiodothyronine and thyroxine were prepared for chromatography as previously described(1), using butanol-acetic and water-collidine-ammonia systems. NaI solution was run separately in the same chromatocab at the same time. Spots were developed with sulfanilic diazonium chloride and palladium chloride for iodide ion. The chromatogram was divided into 1 cm wide strips and each strip counted. The spots were then removed and counted. Urines for each 24 hours were handled in the same fashion. Serum I^{127} determinations were done on samples taken from rats at autopsy.‖

Results. Disappearance of radioactivity from serum of rats given PTU and T4* was linear over 72 hours. The half life of hormone was approximately 18 hours in both iodine deficient and control groups of animals. In Fig.

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‡ Nutritional Biochemicals Corp., Cleveland, O.

§ Radiothyroxine and radiotriiodothyronine were supplied by Abbott Labs., Oak Ridge, Tenn.

‖ Performed in laboratory of Dr. J. Sugarman, Pittsburgh, Pa.