a significant decrease in the diathermy-treated rabbits. ($P = .01$ with 47 degrees of freedom.) All animals in the treated and control groups showed muscle necrosis.

Discussion. Rapid rewarming of frostbitten rabbit legs with diathermy resulted in less extensive necrosis of skin and muscle in comparison to slow thawing in air at room temperature.

The manner in which rapid rewarming of frostbitten tissue is effective in reducing the incidence and extent of necrosis is controversial. We agree with Crismon and Fuhrman (9) that the reduction in the extent of necrosis obtained by rapid rewarming must be due to some action other than vasodilatation. These investigators pointed out that the results from rapid rewarming of frozen rabbit ears were superior to those obtained by stellate ganglion block. The poor results obtained by Finnerean and Shumacker(4) by denervating frostbitten rabbit ears and their reported good results from rapid rewarming suggest the same conclusion. However, Lempke and Shumacker(3) hypothesized that the beneficial results in frozen mice tails obtained by rapid rewarming plus the vasodilatory effect of either tetra-ethyl-ammonium chloride† or sympathectomy are due to the prevention of tissue ischemia and subsequent thrombosis.

It is difficult to interpret these beneficial results attributed to vasodilatation in the light of the favorable results reported by Crismon and Fuhrman(10) from the local application of plaster casts or pressure dressings to frostbitten rabbit feet.

The shortening of the exposure time to cold by the rapid rewarming may be a factor, although Fuhrman and Crismon(2) doubt this explanation since rewarming in water at 36°C did not produce beneficial results in contrast to rewarming at 42°C.

That some metabolic tissue process must be favorably influenced by the local application of heat to the frozen tissue seems to be the best explanation for the beneficial results.

Conclusions. Rapid rewarming of frostbitten rabbit legs by means of short wave diathermy after two standard cold injuries resulted in a decrease in the extent of muscle and skin necrosis in comparison to control frost-bitten animals thawed at room temperature in air.

† Etamon Chloride, Parke, Davis & Co.

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Inhibiting Effect of Inositol on Serum Cholesterol and Phospho-Lipids Following Cholesterol Feeding in Rabbits.*† (19009)

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The role that inositol may play in cholesterol metabolism and in atherosclerosis has not been determined. Herrmann(1) found that inositol lowered the cholesterol in serum, aorta, heart and liver of old hens and(2) in the serum of hypercholesteremic patients. We(3) have found a similar effect in hypercholesteremic diabetic patients, as have Leinwand and Moore(4). On the other hand, Lupton *et al.*(5) found no effect of inositol on

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serum cholesterol levels in diabetic patients; initial levels were not given. Stamler *et al.*(6) found that inositol did not prevent hypercholesteremia and hyperlipemia in cholesterol-fed chicks and(7), did not affect tissue or plasma lipid concentrations or severity of lesions in either spontaneous or stilbestrol induced atherosclerosis. Broun *et al.*(8) found a daily dose of 40 mg of inositol without effect on blood lipids in cholesterol fed rabbits. Davidson *et al.*(9) found inositol did not prevent hypercholesteremia or lesions in dogs fed cholesterol and thiouracil. In view of this controversy, it was decided to determine the effect of inositol on the rise in serum lipids known to occur in cholesterol fed rabbits.

Methods. Young female rabbits, about 4 months old, were maintained during the experimental period on a diet of Purina rabbit chow and hay. In the first experiment, the rabbits were divided into 2 groups. Group A (16 animals) received 1 g of cholesterol, while group B (7 animals) received 1 g of cholesterol plus 0.5 g of inositol daily except Sunday. Both cholesterol and inositol were administered in gelatin capsules which had been previously dipped in molasses to improve palatability as suggested by Pollak(10). After 132 days, some rabbits were sacrificed for pathological examination; the results will be reported later. In the second experiment, the remaining rabbits were given a different feeding regimen for 63 additional days; 11 rabbits from group A were fed 2 g of cholesterol plus 1.2 cc of salad oil, daily except Sunday, and 3 rabbits from group B were

TABLE I. Serum Lipid Values of Rabbits (on Regimens for 132 Days).

	Group*		Days 0	19	35	55	70	91	112	132
Total cholesterol, mg/100 cc	A	Avg	110	130	211	285	387	409	441	481
		Range	71–246	72–340	85–390	120–727	171–1282	223–855	260–871	292–940
	B	Avg	83	83	88	93	113	119	120	226
		Range	68–107	68–111	63–120	66–136	85– 144	99–147	104–162	106–855
Cholesterol esters, mg/100 cc	A	Avg	80	95	154	203	279	290	312	334
		Range	52–180	52–267	62–299	86–541	116–1015	160–616	178–621	193–663
	B	Avg	59	59	62	64	78	81	82	154
		Range	48– 72	49– 69	44– 74	47– 88	61– 125	68–113	68–108	70–577
Ratio, chol. est./ tot. chol.	A		.73							.69
	B		.72							.69
Lipid phosphorus, mg/100 cc	A	Avg	4.2	4.6	6.5	7.9	9.3	9.7	9.9	10.3
		Range	2.3–6.3	2.5–9.4	3.4–10.3	4.6–15.6	5.3–20.6	5.4–18.4	5.6–18.4	5.8–18.8
	B	Avg	4.1	4.2	4.4	4.5	4.7	4.7	4.7	5.2
		Range	3.1–5.4	3.1–5.5	3.4– 5.6	3.3– 6	3.5– 6.1	3.8– 6.2	3.9– 6.2	3.9– 8.8
Molar ratio, Tot. chol./lip. phos.	A		2.13							3.75
	B		1.70							2.14

* The 16 rabbits in Group A received cholesterol, the 7 in Group B cholesterol + inositol.

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TABLE II. Serum Lipid Values on Rabbits (on Regimens for 63 Days).

	Group*		Value at start	Days 21	Days 42	Days 63
Total cholesterol, mg/100 cc	A	Avg	424	564	929	1050
		Range	292–628	372–823	710–1340	748–1563
	B	Avg	190	198	338	370
		Range	110–323	119–333	202– 427	217– 500
Cholesterol esters, mg/100 cc	A	Avg	288	392	659	739
		Range	193–443	259–577	500– 941	518–1136
	B	Avg	134	138	235	252
		Range	75–233	78–241	134– 307	147– 336
Lipid phosphorus, mg/100 cc	A	Avg	8.7	9.9	14.7	16.3
		Range	5.8–11	6.6–15.5	10.8–18.1	12.2–25
	B	Avg	6.3	6.4	8.2	8.6
		Range	4–8.8	4.2– 8.8	6.6– 8.8	6.7– 9.5

* The 11 rabbits in Group A received cholesterol and salad oil, the 3 in Group B these components + inositol.

given these dietary supplements plus 1 g of inositol. An inanition period of 18 hours preceded all blood sampling. Blood specimens were obtained from the marginal ear vein after hyperemia was produced by application of a small amount of xylene. On the day prior to the initial feeding of cholesterol and inositol and at approximately three-week intervals thereafter, blood specimens were obtained for the determination on serum of total cholesterol and cholesterol esters by the method of Sperry and Brand(11), and lipid phosphorus by the method of Youngburg and Youngburg (12), using the phosphate procedure of Fiske and SubbaRow(13).

Results. In the first experiment, weight increases were similar in both groups, averaging 70% and 68% during 132 days for group A and B, respectively.

The rise in serum lipid components studied for both groups is shown in Table I. The sudden rise in the average of all lipids in group B on the 132nd day is due to a sudden inexplicable increase in the values of one animal. During the 20 day period, this animal showed a rise (which was rechecked on another day) of 749 mg in total cholesterol, 504 mg in cholesterol esters and 3.6 mg in lipid phosphorus, while the other rabbits in group B showed no significant rise. It would appear that under the conditions of this experiment, inositol almost completely inhibits the rise in serum lipids following cholesterol feeding, except in the case of one rabbit at 132 days.

The ester/total cholesterol ratio showed no significant change over the course of the experiment in either group, but the molar ratio of total cholesterol to lipid phosphorus increased more in group A (76%) than in group B (26%).

Table II shows that the results of the second experiment are qualitatively the same as in the first experiment. With this dietary regimen inositol was apparently not as effective in preventing a further rise in serum lipids. This may be due to the fact that the rabbits started at a higher lipid level than in the first experiment, that they received extra lipid (as salad oil) in their diet, or to the possibility that the amount of inositol required for inhibition may increase as the cholesterol intake increases.

Conclusions. From the above experiments, it would appear that inositol inhibits the expected rise in cholesterol and phospholipid levels in serum of rabbits on high cholesterol diets.

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