tion of the alpha-cells with cobaltous chloride and the blockade of sympathetic effects with DHE (or related substances) should lend itself to a study of the mechanism of blood sugar control and the specific need for these hyperglycemic agents under varied conditions.

Summary. 1. DHE did not interfere with the glycogenolytic effect of HGF on rabbit liver slices or on hyperglycemia in intact rabbits. Comparable effects of epinephrine *in vitro* or *in vivo* were antagonized by DHE. 2. The hyperglycemia induced by cobaltous chloride, which destroys alpha-cells of the pancreatic islets, was prevented by DHE. This hyperglycemia is attributable to sympathetic nerve stimulation. No evidence for the release of HGF was obtained. 3. Blood sugar was maintained at a normal level in rabbits pretreated with cobalt, then fasted and injected with DHE to limit sympathetic action on the liver.

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1. Goldner, M. G., Volk, B. W., and Lazarus, S. S., *Metabolism,* 1952, v1, 544.
2. Avezzu, G., *Arch. E. Margliano patol. e. clin.,* 1952, v7, 837.
3. Sutherland, E. W., *Ann. N. Y. Acad. Sci.,* 1951, v54, 693.
4. Ellis, S., Anderson, H. L., Jr., and Turtle, M. A., *J. Pharm. Exp. Therap.,* 1952, v106, 383.
5. Sutherland, E. W., and Cori, C. F., *J. Biol. Chem.,* 1948, v172, 737.
6. Nelson, H., *ibid.,* 1944, v153, 375.
7. Staub, A., Sinn, L., and Behrens, O. K., *Proc. XIX International Physiol. Cong.,* Montreal, 1953, p797.

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Effect of Inositol Feeding on Inositol Phosphatides and Other Lipids of Human Blood Plasma.* (20654)

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Concerned by the role of atherosclerosis in producing hearing loss and labyrinthine dysfunction, it occurred to one of us that a lipotropic factor might be of some clinical value. A study of such possible effects of inositol was initated and has continued for the past several years. Biochemical as well as clinical methods were included. A method has been developed for the determination of lipid inositol in various tissues including blood plasma (1). Results of the determination of the effect of inositol ingestion on the concentration of plasma lipid inositol and several other plasma lipids are summarized in this paper.

Methods. All of the patients reported in this study showed varying degrees of high tone deafness and ranged from 34 to 68 years of age. One initial blood sample was obtained before oral inositol was started. The values for the various lipid components in these samples are in the normal range for the most part and the group could not be considered lipemic. The patients then all received 1 g of inositol† 3 times daily. Blood samples were obtained at varying intervals thereafter and averaged 2 samples per patient. The duration of inositol feeding in the patients reported ranged from 1 to 12 months. None of the patients reported any untoward symptoms or reactions from the inositol. All blood samples were obtained before breakfast between 9 and 9:30 a.m. 50 ml of blood was withdrawn into a centrifuge bottle containing 2 ml of Wintrobe's oxalate. The lipid extracts of the plasma were prepared, purified, and analyzed for total lipid phosphorus and inositol by methods reported previously(1,2). Total fatty acids were determined titrametrically by a modification of the method of Stoddard and Drury(3) on the purified extract.

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† The inositol was generously supplied by the Corn Products Refining Co., Argo, Ill.

TABLE I. Effect of Inositol Feeding on Lipids of Human Blood Plasma in Thirteen Patients.

	Mean		Range		Mean			
	Initial	Final	Initial	Final	change	S.E.	t	P
Total lipid P	295 *	310	230-389	244-376	+ 5.30 ‡	2.12	2.49	.031
Total lipid inositol	7.4*	7.3	5.5-9.4	4.5-9.8	— .8 ‡	2.82	.28	.8
Lipid inositol:phosphorus molar ratio	.025	.024	.022-.029	.019-.029	— 5.44 ‡	2.40	2.27	.04
Total cholesterol	223 †	242	160-309	183-319	+ 9.63 ‡	3.90	2.47	.033
Free cholesterol	61.9†	67.4	42.2-88.8	48.6-91.3	+ 10.0 ‡	3.60	2.78	.017
Ratio free:total cholesterol	.276	.277	.249-.291	.258-.300	+ .50 ‡	1.23	.408	.7
Ratio total cholesterol: lipid P	.757	.785	.624-.913	.689-1.078	+ 4.23 ‡	3.26	1.30	0.2
Neutral fat fatty acids	349 *	161	71-1054	13-374	—188 §	68.2	2.76	.02
Ratio neutral fat F.A.: total cholesterol	1.48	0.67	0.38-3.98	0.07-1.56	— .806§	.259	3.11	.01

* Values given in μm/100 ml plasma. † Values given in mg/100 ml plasma. ‡ Mean % change. § Mean numerical change.

Total and free cholesterol were determined on a separate aliquot of the plasma by the method of Schoenheimer and Sperry(4).

Results. A summary of the results obtained from 13 patients is given in Table I. In this summary the analyses of the last sample only after inositol feeding is chosen for comparison with the initial pre-inositol blood since it represents the maximum time of inositol intake. Cholesterol (free and total), total phospholipids and inositol phospholipids are calculated as the percent change from the initial sample. Neutral fat fatty acids are a more variable component and are therefore calculated in terms of absolute change in micromoles per cent. The statistical method for evaluation of the data is that of Fischer in which Standard Errors of the Difference are obtained using the initial value as zero. The "t" values are thus obtained as the change from the initial sample divided by the Standard Error of the Difference.

Discussion. Although the series is small it seems established that no real change occurred in the concentration of plasma inositol phosphatides incident to feeding amounts of inositol which are many times greater than the ordinary dietary intake. The ratio of inositol phosphatides to the total plasma phosphatides appears to be quite constant and all the values fall within the range of .019 to .029 in the samples reported. In another group of 6 patients who did not receive inositol and are not reported, the values also conformed to this range.

There appeared to be small but measurable mean increases in total cholesterol, free cholesterol, and total phospholipids. However, these changes are of doubtful statistical significance. These latter lipid components have been measured in several inositol feeding experiments on man and animals by others who reported (a) decreases in total cholesterol (5-8) and phospholipids(7) or (b) little or no change in cholesterol(9-12), phospholipids (10,12) and low density lipoproteins(12). In cholesterol fed rabbits, inositol was reported to be both ineffective(13) and effective(14) in preventing the hypercholesteremia. In old hens inositol reduced cholesteremia(15) but failed in combination with choline to reduce the cholesteremia and general lipemia in chicks fed cholesterol(16) or treated with estrogen (17). It seems clear that no simple relationship, if any, exists between exogenous inositol and blood plasma total cholesterol, free cholesterol or total phospholipids.

In this study, the neutral fat fatty acids decreased with inositol feeding, and more strikingly in the more lipemic patients—*i.e.*, those with initial cholesterol values over 200 mg%. The decreases as recorded appear to be significant. However, caution must be exercised in the interpretation of changes in as highly variable a fraction as the neutral fat. The mean decrease in the "lipemic" group is less than the magnitude of what must be considered a "normal" range for neutral fat. Moreover, no consistant decrease in the neutral fat fraction was observed in cholesterol fed chicks(15) or estrogen treated chicks(16) given inositol with choline. Nevertheless,

these data are of considerable interest since at least a part of this fraction is associated with low density lipoproteins(18) which in turn are thought by some to be associated with the development of atherosclerosis. Studies are in progress to further examine such a relationship between inositol and neutral fat.

Summary. A group of 13 patients with high tone deafness was treated with 3 g oral inositol daily for periods of 1 to 12 months. No change resulted in the fasting plasma inositol phosphatide concentration; there were minor increases in total and free cholesterol and total phospholipids, and there was an apparent decrease in neutral fat.

1. Taylor, W. E., and McKibbin, J. M., *J. Biol. Chem.*, 1953, v201, 609.

2. ———, *ibid.*, 1951, v188, 677.

3. Stoddard, J. L., and Drury, P. E., *ibid.*, 1929, v84, 741.

4. Schoenheimer, R., and Sperry, W. M., *ibid.*, 1934, v106, 745.

5. Herrmann, G. R., *Exp. Med. and Surg.*, 1947, v5, 149.

6. Felch, W. C., and Dotti, L. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 376.

7. Felch, W. C., Keating, J. H., and Dotti, L. B., *Am. Heart J.*, 1952, v44, 390.

8. Sherber, D. A., and Levites, M. M., *J. A. M. A.*, 1953, v152, 682.

9. Lupton, A. M., Battafarano, T. W., Murphy, F. E., and Brown, C. L., *Ann. Western Med. and Surg.*, 1949, v3, 342.

10. Leinwand, I., and Moore, D. H., *Am. Heart. J.*, 1949, v38, 467.

11. Popkin, R. J., *Angiology*, 1951, v2, 398.

12. DeWind, L. T., Michaels, G. D., and Kinsell, L. W., *Ann. Int. Med.*, 1952, v37, 344.

13. Moses, C., Rhodes, G. L., and Delacio, A, *Angiology*, 1952, v3, 238.

14. Dotti, L. B., Felch, W. C., and Ilka, S. J., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 167.

15. Herrmann, G. R., *ibid.*, 1946, v63, 436.

16. Stamler, J., Bolene, C., Harris, R., and Katz, L. N., *Circulation*, 1950, v2, 714.

17. ———, *ibid.*, 1950, v2, 722.

18. Jones, H. B., Gofman, J. W., Lindgren, F. T., Lyon, T. P., Graham, D. M., Strisower, B., and Nichols, A. V., *Am. J. Med.*, 1951, v11, 358.

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Vitamin E Activity of Alpha-Tocopherylhydroquinone and Muscular Dystrophy. (20655)

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It was shown for the first time some 14 years ago that the muscular dystrophy produced in rabbits fed certain natural food diets, purified fat free diets, or cod liver oil, can be prevented or cured by highly purified concentrates of wheat germ oil or by α-tocopherol(1-3). The effect of vit. E on the intense creatinurea exhibited by dystrophic rabbits was dramatic, a precipitous fall in creatine excretion occurring in the first 24 or 48 hours after the initiation of therapy(1). Subsequently it was found that the duration of the fall in creatine excretion caused by single doses of α-tocopherol provided an excellent bioassay for antidystrophic activity, the number of days of suppressed creatinurea being proportional to the size of the dose administered(4). Utilizing this antidystrophic assay in rabbits, it was discovered recently that α-tocopherylhydroquinone is a potent antidystrophic substance(5,6). Indeed the antidystrophic activity of a 5 mg dose given intravenously approximates the activity of α-tocopherol itself. However, the antidystrophic activity of the hydroquinone is somewhat unique, for unlike α-tocopherol it is more potent when administered intravenously than when given orally. Moreover, unlike α-tocopherol, it is not stored, for increas-

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