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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ing the dose of α-tocopherylhydroquinone beyond a 5 mg level does not appreciably extend the duration of the response.†

These findings suggested to us(6) that α-tocopherylhydroquinone possesses antidystrophic properties *per se,* rather than as a result of its conversion to α-tocopherol in the body. In an attempt to shed further light on this hypothesis, we have investigated the antisterility activity of α-tocopherylhydroquinone in vit. E deficient female rats. Since the hydroquinone is not stored, it was evident that the administration of a single dose at the beginning of pregnancy would not be satisfactory and that repeated injections should be given throughout pregnancy(6). However, the rapidity with which the free hydroquinone undergoes auto-oxidation presented difficulties for such a procedure. Consequently the more stable α-tocopherylhydroquinone disuccinate, which has also been shown to possess antidystrophic activity(7), was employed. This derivative was repeatedly injected via the tail vein throughout pregnancy. The antisterility activity of α-tocopherol administered intravenously was likewise examined. The effect of injected α-tocopherylhydroquinone and its disuccinate on the level of serum tocopherol in dystrophic rabbits was also determined. These experiments and their implications with respect to the *in vivo* activity of α-tocopherylhydroquinone comprise the subject of this paper.

Methods. Female rats of the Sprague-Dawley strain were placed on the vit. E deficient diet of Mason and Harris(8) at the time of weaning. Each rat received 2 drops of percomorph oil orally 3 times a week, and 0.5 mg of a vit. K preparation (Synkamin) intraperitoneally once a week. After the young rats had been maintained on this regimen for 4 months they were subjected to an initial resorption gestation. Following a period of recovery, the animals were placed with a male at Stage I of estrus, and test compounds were injected on the following day when sperm were found in the vaginal smear. Prior to injection, each rat was placed before an infrared lamp for 15 minutes. The animal was then immobilized by wrapping it snugly in a double length of cheesecloth, and its tail was immersed in a water bath at 40-45°C for 3 minutes, following which the test compound was injected into the dilated tail vein through a No. 25 gauge needle. Unless these precautions to insure complete dilatation of the tail vein were taken, cellulitis and necrosis of the tail resulted, rendering the animal unsuitable for further supplementation.

The sodium disuccinate of α-tocopherylhydroquinone,‡ prepared according to the procedure described earlier(7), was dissolved in either 0.15 M phosphate buffer or in isotonic saline and adjusted to pH 7.4. The volume of a single injection of these disuccinate solutions did not exceed 1.5 ml. Alpha-tocopherol was administered as a suspension in one part of ethanol and 9 parts of propylene glycol. Although the propylene glycol present in as little as 0.05 ml of this mixture produced hematuria within 30 minutes of the intravenous injection, this reaction did not interfere with pregnancy. In all tests the appearance of the placental sign between the 10th and 15th day following insemination and the typical gain in weight were both required as evidence of pregnancy.

The antidystrophic activity of the disodium disuccinate of α-tocopherylhydroquinone was checked by intravenous administration to vit. E deficient dystrophic rabbits. The succinate was again found to have from one-quarter to one-half of the antidystrophic activity, on a molar basis, of the free hydroquinone or of α-tocopherol(7). Serum tocopherol levels were determined by the method of Quaife, Scrimshaw, and Lowry(9).

Results and discussion. In the initial experiments female rats with no detectable toco-

† Frequent administration of small doses of α-tocopherylhydroquinone completely presents or cures dystrophy in the rabbit(5,6). These findings on the rabbit have been extended by Dr. Karl Mason to the prevention and cure of dystrophic lesions in the suckling rat and in the adult hamster by α-tocopherylhydroquinone (Personal communication).

‡ We are indebted to Dr. Milton Farber and Dr. Ade T. Milhorat for generous supplies of α-tocopherylhydroqinone disodium disuccinate and α-tocopherylhydroquinone.

TABLE I. Antisterility Activity of α-Tocopherylhydroquinone Disodium Disuccinate and α-Tocopherol Injected Intravenously in Vit. E-Deficient Female Rats during Pregnancy.

No. of rats	Supplement	mg per dose	No. of doses	Days between doses*	Total mg inj.	No. of rats delivering
5	α-T.hydroquinone disucc.†	8	6	2	48	0
4	" "	8	10	1	80	0
8	" "	4	16	0	64	0
8	" "	8	16	0	128	0
6	d,l α-Tocopherol‡	3	1		3	6
9	α-T.hydroquinone disucc. + d,l α-Tocopherol	8 3	19 1	0	152 3	9

* First dose of test compound was inj. on day that sperm appeared in vaginal smear.

† α-tocopheryllhydroquinone disodium disuccinate. 8.04 mg of this material are equivalent on a molar basis to 5.0 mg of α-tocopherol.

‡ Kindly supplied by Merck and Co., Rahway, N. J.

pherol in their serum were injected by tail vein with 8 mg of α-tocopherylhydroquinone disodium disuccinate on every third day or on alternate days throughout pregnancy. In neither case was this treatment effective and typical resorption pregnancies resulted (Table I). In the next experiment 8 similar rats were injected daily with 4 mg of the disuccinate. By the end of the 16th day it was evident from the sharp decline in the weight curves that resorption of the young had occurred in each of these animals. After a period of rest these same 8 rats were mated again and injected with 8 mg of the disuccinate *daily* for 16 days, or with a total of 128 mg, equivalent, on a molar basis, to 80 mg of α-tocopherol. None of these animals gave birth to young (Table I) and all showed the loss of weight, beginning at about the 16th day, that is typical of resorption.

In contrast to the results obtained with the hydroquinone the intravenous administration of a single 3 mg dose of α-tocopherol on the first day of pregnancy resulted in the birth of living young to each of the rats tested (Table I). Moreover, when the rats who had failed to respond to the hydroquinone were injected with a single dose of α-tocopherol at the beginning of a subsequent pregnancy, living young were born in every instance. Finally, vit. E-deficient rats were given a 3 mg dose of α-tocopherol by mouth on the day of insemination and were then injected daily with 8 mg of the disuccinate for the next 19 days (Table I). All of these rats gave birth to living young, thus eliminating the possibility that trauma caused by repeated injections was responsible for the negative findings obtained with the hydroquinone. The results of these experiments show that α-tocopherylhydroquinone disuccinate is essentially devoid of antisterility activity when administered under the same conditions that led to the discovery of its antidystrophic activity.

It may be calculated on the basis of the foregoing experiments that less than one-tenth of the α-tocopherylhydroquinone disuccinate could have been converted to α-tocopherol in the animal body. Mason(10) has shown that the administration of divided doses of vit. E during the first 10 days of pregnancy is a very satisfactory procedure, and that 0.7 mg of α-tocopherol so administered prevents sterility in a large number of rats. Our rats which were injected with 8 mg of the disuccinate daily received during the first 10 days a quantity of α-tocopherylhydroquinone equivalent on a molar basis to 50 mg of α-tocopherol, yet not one of them gave birth to young. In terms of antidystrophic activity, this amount of the disuccinate is equal to 12-25 mg of α-tocopherol. Consequently it appears that the antidystrophic activity of α-tocopherylhydroquinone cannot be accounted for by its conversion to α-tocopherol in the body.

Additional support for this conclusion was obtained in a study of the level of serum tocopherol produced by the intravenous administration of α-tocopherol, α-tocopherylhydroquinone, and the disuccinate of the latter compound to rabbits with no detectable

TABLE II. Serum Tocopherol 24 Hours after Intravenous Injection of Single Doses of Antidystrophic Compounds in Rabbits with Stage I Dystrophy and No Detectable Serum Tocopherol.

Compound	mg inj.	Serum tocopherol, mg %
d,l α-Tocopherol	5	.5
"	10	1.2
"	10	1.1
"	10	1.0
"	10	1.1
α-Tocopherylhydroquinone	10	0
"	10	0
"	100	0
α-T.-hydroquinone disucc.*	20	0
" "	20	0
" "	40	0
" "	100	0

* α-tocopherylhydroquinone disodium disuccinate.

serum tocopherol. As shown in Table II, when such animals were injected with a single dose of 5 or 10 mg of α-tocopherol, substantial levels of tocopherol were found in the serum 24 hours later. In contrast to these results, the injection of even 10 times as much hydroquinone or hydroquinone disuccinate gave rise to no detectable serum tocopherol. Nevertheless, the latter compounds produced a drop in creatine excretion and marked improvement in the physical symptoms of dystrophy.

Both the results of these latter experiments and of the antisterility assay experiments indicate that α-tocopherylhydroquinone is not converted to α-tocopherol to a significant extent in the body. It appears therefore that α-tocopherylhydroquinone possesses antidystrophic activity *per se*, and is, in effect, an antidystrophic vitamin. The separation of antidystrophic from antisterility activity is a matter of considerable interest for the most ubiquitous response of experimental and domestic animals to vit. E-deficiency is muscle degeneration. This is true even in the rat, our classic and overemphasized example of sterility, for at both extremes of its life span the rat develops severe muscle lesions in the face of vit. E deprivation, and in the interim it is afflicted with muscle weakness and scattered muscle lesions(11). Many other vit. E-deficient animals develop dystrophy in the absence of signs of sterility. For example, rabbits subjected to repeated attacks of muscular dystrophy and finally allowed to succumb to the disease, do not develop testicular degeneration(12,13) and female rabbits with extensive muscle lesions give birth to living but dystrophic young(14).

The dystrophy caused by vit. E deficiency may be prevented or cured by α-tocopherylhydroquinone, which is devoid of antisterility activity; or, by α-tocopherol, which exhibits both antisterility and antidystrophic activity. With respect to the antidystrophic effect of α-tocopherol, 2 possibilities exist; first, that it possesses antidystrophic activity as such, or second, that its antidystrophic activity is due to its conversion in the body to the hydroquinone and that, with respect to this function, it is a provitamin or storage form for the active antidystrophic factor, α-tocopherylhydroquinone. If the second alternative is indeed the case, muscular dystrophy could be caused by any one of several conditions: a deficiency of vit. E, an inability to convert tocopherol to the active hydroquinone, or excessive destruction *in vivo* of this unstable compound. In view of these possibilities and the finding that α-tocopherylhydroquinone is an antidystrophic substance in its own right, the need for a critical and sober examination of its effect on human muscular dystrophies is clearly indicated.

Summary and conclusions. The disuccinate of α-tocopherylhydroquinone has been tested for antisterility activity by daily intravenous injections in pregnant vit. E-deficient rats. This antidystrophic compound was found to be devoid of the antisterility activity associated with α-tocopherol. Moreover, the injection of dystrophic rabbits with large curative doses of α-tocopherylhydroquinone, or its disuccinate, did not lead to the appearance of tocopherol in the serum. Both of these observations indicate that α-tocopherylhydroquinone is not converted to α-tocopherol to a significant extent in the body. It appears therefore that α-tocopherylhydroquinone is an antidystrophic factor or vitamin *per se*. Alpha-tocopherol, on the other hand, is the antisterility vitamin. It may possess antidystrophic activity as such, or alternatively, it may only be the provitamin for α-tocopherylhydroquinone. The separation of anti-

dystrophic from antisterility activity is discussed with relation to the function of vit. E in muscular dystrophy.

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1. Mackenzie, C. G., and McCollum, E. V., *Science,* 1939, v89, 370; *J. Nutrition,* 1940, v19, 345.

2. Mackenzie, C. G., Mackenzie, J. B., and McCollum, E. V., *J. Nutrition,* 1941, v21, 225.

3. ———, *Science,* 1941, v94, 216.

4. Mackenzie, C. G., *Fed. Proc.,* 1942, v1, 190.

5. Mackenzie, J. B., Rosenkrantz, H., Ulick, S., and Milhorat, A. T., *J. Biol. Chem.,* 1950, v183, 655.

6. Mackenzie, J. B., *Biological Antioxidants, Transactions of the Fourth Conference,* 1949. Edited by C. G. Mackenzie, New York, Josiah Macy, Jr., Foundation, p174, also discussion p177.

7. Farber, M. J., Mackenzie, J. B., Rosenkrantz, H., and Milhorat, A. T., *J. Biol. Chem.,* 1951, v190, 445.

8. Mason, K. E., and Harris, P. L., *Biol. Symposia,* 1947, v12, 495.

9. Quaife, M. L., Scrimshaw, N. S., and Lowry, O. H., *J. Biol. Chem.,* 1949, v180, 1229.

10. Mason, K. E., *Am. J. Physiol.,* 1940, v131, 268.

11. Mackenzie, C. G., *Ann. N. Y. Acad. Sci.,* 1949, v52, 202.

12. Mackenzie, C. G., and McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1941, v47, 148.

13. Mackenzie, C. G., *ibid.,* 1942, v49, 313.

14. Pappenheimer, A. M., and Goettsch, M., *ibid.,* 1936, v34, 522.

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The Precipitability of Two Modified Proteins.* (20656)

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Precipitin-type reactions, exhibiting specificity between iodinated proteins and arsonobenzeneazoproteins, have been reported for extracts of *Saccharomyces cerevisiae* repeatedly cultured in the presence of such modified proteins(1). From extended attempts to extrapolate this work, it has been learned that such precipitates may be obtained from complex combinations of experimental conditions, in the absence of the yeast. The specificity which was originally observed is believed, therefore, to be non-biological in all or most of the cases studied. That a major factor contributing to the observed precipitin-like reactions and their variation may have been pH was suggested by the fact that precipitin-like behavior was frequent during some periods of experimentation, yet infrequent in others. After many such periods, the former type appeared to be correlative with higher acid content in the samples of ether employed in cytolysis. The finding that some cultures yielded more reactive extracts was probably due to fortuitous acid content rather than to a parallelism between behavior of yeast and the long-known erratic antibody-producing power of animals(2).

Haurowitz(3) has shown that reported instances of precipitin reactions from *in vitro* incubation of serum globulin with acid azoovalbumin(4) is ascribable predominantly to charge effects. In the case of the yeast cultures, however, the more rigorous test of specificity of reaction appeared to be met. The results reported in this paper suggest that such results may appear when the interplay of conditions leads to overlapping, yet in part discrete, pH regions of precipitability for two or more antigens. In the course of these studies, attention was particularly focussed upon the pH factor by the related finding that evaluation of specificity of protease-substrate interaction was dependent upon pH to a degree much greater than that earlier recognized (5-9). In both types of situation, other factors than pH contribute to the effects observed.

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