

slicing is to be done in the open, but need not be moistened in the humid box of Sperry (2) or the moist cold box of Fuhrman and Field (1).

There is no apparent change in the tissues induced by the low frequency vibration of the blade, as measured by the 1-hr oxygen uptake of comparative portions of the same organ sliced by hand (4) and by the vibrating cutter, as shown in Table 1. The medium used was Krebs's Phosphate Ringer's, pH 7.4, at 37° C, with air atmosphere. The brain medium also contained .011 molar glucose.

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## The Chemical Nature of a Factor in Hog Stomach Extracts that Reduces the Creatinuria of Muscular Dystrophy<sup>1</sup>

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A labile factor, assayed by its effect on lowering the creatinuria of a patient with progressive muscular dystrophy, has been found to concentrate in the fat fraction (7) of hog gastric mucin<sup>2</sup> and of hog stomach linings<sup>3</sup>. But attempts at further fractionation of this concentrate by countercurrent distribution between absolute methanol and isooctane led to a rapid loss of biological activity. The crude fat fraction contained up to 1%<sup>4</sup> of a substance which reduced ferric chloride in a modified Emmerie and Engel (1) assay. This reducing property proved to be a measure of biological activity, since loss in biological activity was associated with a decrease in amount of reducing substance. None of the four naturally occurring tocopherols, which also reduce ferric chloride in the assay of Emmerie and Engel, could have accounted for the biological activity, since they were without effect on the creatinuria of this patient at doses

25 times the active dose of reducing substance from stomach extracts. Alpha (6), beta, and gamma tocopherols (4), however, do abolish the creatinuria of the muscular dystrophy of experimental vitamin E deficiency.

The fat fraction of hog gastric mucin which lowered creatinuria at a dosage of 100 mg was allowed to lose activity by standing at room temperature for 3 months. The amount of reducing substance fell from 0.77% to 0.07% and biological activity was completely lost. This suggested a destruction by autoxidation which leads, in the case of tocopherols in the presence of fats, to the formation of tocopheryl-*p*-quinones (6). The tocopheryl-*p*-quinones, in turn, can be reduced to the corresponding tocopheryl-*p*-hydroquinones and cyclized in the presence of mineral acid to regenerate the original tocopherols (5). The following experiments, which were designed to regenerate any tocopherols in the inactivated fat fraction that had undergone autoxidation and tocopheryl-*p*-quinone formation, led to the finding that an intermediate in the reaction, a simple reduction product, possessed chemical and biological properties similar to the factor in the fat fraction of hog gastric mucin. A 100-mg portion of inactivated fat fraction was refluxed for 2 hr in an isooctane-ethanol mixture containing 5.0 g stannous chloride and 5.0 ml concentrated HCl. After addition of water and recovery of the isooctane layer, the amount of reducing substance was found to have increased to 1.08% and remained stable at this value. A 225-mg dose of this material containing 2.43 mg of reducing substance had no effect on creatinuria. A control experiment with pure alpha tocopheryl-*p*-quinone under the same conditions showed complete conversion to alpha tocopherol ( $E_{1\text{ cm}}^{1\%}$  [298 mμ] in isooctane = 70;  $E_{1\text{ cm}}^{1\%}$  [520 mμ] in the Emmerie and Engel assay = 370). Another 100-mg portion of inactivated fat fraction of gastric mucin was refluxed for 30 min in the same solvent with 0.7 g stannous chloride and 0.5 ml concentrated HCl, and the isooctane layer was recovered as described. The reducing substance had increased to 0.73% but fell to 0.30% on the second day, 0.27% on the third day, and to 0.21% by the end of a week. A 225-mg dose of this material, containing 1.03 mg of reducing substance, was biologically active. The control with pure alpha tocopheryl-*p*-quinone under these conditions showed the formation of alpha tocopheryl-*p*-hydroquinone by the appearance of an absorption maximum at 290 mμ which disappeared in the course of 18 hr, as the spectrum of alpha tocopheryl-*p*-quinone reappeared with a double maximum between 260 and 270 mμ. This rapid autoxidation is characteristic of alpha tocopheryl-*p*-hydroquinone, first described by John (5). The creatinuria-lowering activity of the fraction of gastric mucin treated in this way was fully duplicated by 1.0 mg of pure synthetic alpha tocopheryl-*p*-hydroquinone.

Alpha tocopheryl-*p*-hydroquinone was prepared as needed from the more stable alpha tocopheryl-*p*-quinone by catalytic hydrogenation in ethanol or propylene glycol with palladium on calcium carbonate. The  $E_{1\text{ cm}}^{1\%}$  (290 mμ) in isooctane was 88 immediately after hydrogenation, a value somewhat higher than that reported by John (5). The  $E_{1\text{ cm}}^{1\%}$  (520 mμ) in the Emmerie and Engel assay was

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<sup>2</sup> Frederick Stearns and Company. Prepared by alcoholic precipitation of an acid digestion of hog stomach linings.

<sup>3</sup> A low temperature concentrate of an acetone extract of hog stomach linings was kindly furnished by the Armour Research Laboratories.

<sup>4</sup> Calculated from a standard curve for alpha tocopherol.

390. Alpha tocopheryl-*p*-quinone was prepared by the oxidation of natural or synthetic alpha tocopherol with ferric chloride (2) and purified by the method of Tishler and Wendler (3) to an  $E_{1\text{ cm}}^{1\%}$  (268 m $\mu$ ) in iso-octane of 450.

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### Taste Blindness to Phenyl-Thio-Carbamide as a Function of Saliva

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In 1931, A. L. Fox (6, 7) of the E. I. du Pont de Nemours Company discovered that approximately 60% of the Causasian population are able to taste phenyl-thio-carbamide (P. T. C.) as bitter, while the remaining 40% find it to be as tasteless as chalk. The literature of the physiological, genetic, environmental, and ethnological characteristics of this phenomenon has been reviewed by Cohen and Ogdon (3).

Blakeslee (1), Fox (4), Mee (5), and Blakeslee and Salmon (2) have all suggested that it may be the saliva which is the determiner of the ability to taste or not, and not the "taste apparatus" itself. Fox specifically suggested the possibility that nontasters have in their saliva a product, possibly a protein or a colloid, which precipitates the P. T. C. as a very insoluble substance which does not give rise to a taste sensation.

The question as to the effect of the saliva may be settled by allowing tasters and nontasters to taste P. T. C. using another taster's or nontaster's saliva. A population of 35 American college students was first tested with P. T. C. crystals and each individual classified as a taster or a nontaster. Then each person (whether taster or nontaster) was tested with each of the following tests: 1) D test, using a saturated solution of P. T. C. in tap water on a dry (dried by air from an atomizer) tongue; 2) G test, using a saturated solution of nontaster's saliva on a dry tongue; 3) P test, using a saturated solution of P. T. C. in taster's saliva on a dry tongue. The taster's and nontaster's saliva was obtained from other individuals selected purely at random. No saliva from any person was used for more than one test on more than one subject. The results are given in Table 1.

As a control, 19 individuals were tested with the R test, using saturated solutions of P. T. C. in their own

saliva, which had been allowed to remain exposed to air about 5-10 min, on a dry tongue. The results are given in Table 2.

TABLE 1

SENSATIONS OF 35 OBSERVERS TO P. T. C. DISSOLVED IN WATER, AND TASTER'S AND NONTASTER'S SALIVA ON A DRY TONGUE

|              | D Test       | G Test        | P Test       |
|--------------|--------------|---------------|--------------|
| 26 Tasters*  | T=0<br>NT=26 | T=0<br>NT=26† | T=0<br>NT=26 |
| 7 Nontasters | T=0<br>NT=7  | T=0<br>NT=7   | T=0<br>NT=7‡ |

T = tastes bitter.

NT = no taste.

\* Four subjects reported only weak taste of bitter.

† One subject reported slight sensation, but was unable to describe it.

‡ One subject reported a "warm" sensation.

These data seem to indicate that an individual will taste P. T. C. as bitter when the following two necessary conditions are met: 1) He must have the correct "taste apparatus," and 2) he must have his own saliva (or,

TABLE 2

SENSATIONS OF 19 OBSERVERS TO P. T. C. DISSOLVED IN THEIR OWN SALIVA ON A DRY TONGUE

|              | R Test        |
|--------------|---------------|
| 17 Tasters   | T=16<br>NT=1* |
| 2 Nontasters | T=0<br>NT=2   |

\* This subject had previously reported a weak bitter taste to the crystals.

presumably, its chemical equivalent). A nontaster cannot taste in any event, even when he uses the saliva of another taster. A taster cannot taste under any circumstances, except when he uses his own saliva; he cannot taste if he uses the saliva of another taster or nontaster. He can taste if he uses his own saliva, even though the saliva is placed on his tongue in exactly the same manner as the saliva from another individual. No subject can taste P. T. C. when the crystals are dissolved in water and no saliva is used at all.

Salivas are probably as different as fingerprints. Blakeslee and Salmon (2) have also found that saliva is important to taste P. T. C. Our finding that it must be the individual's own saliva may be brought about by the fact that the "taste apparatus" becomes, over the years, extremely sensitive and specialized to the particular saliva which the individual possesses; or these differences in saliva may be congenital or genetic. This being the case, when other saliva is introduced, it is equivalent to water, and no taste sensation results.

As a check on whether saliva influences other tastes, 27 observers were tested with saturated solutions (in water) of saccharin and salt on dry tongues, and on tongues wet with saliva. All of the individuals were able