variations in gonad-stimulating potency exist in the pituitaries of castrated as compared with normal newts and of males as compared with females. If all of the series had been killed after five or six grafts, thus utilizing the criterion of ovulation rather than egg-laying, the data might have been more conclusive. It is also possible that gonadectomy in the fall of the year when the gonads are already mature may have relatively little effect on the gonad-stimulating hormonal content of the pituitaries as compared with possible effects in the summer when active spermatogenesis and oogenesis are in progress. This should be determined. But even in the fall, the already matured gonad is dependent for maintenance on the pituitary as the effects of hypophysectomy indicate, so that some gonad-stimulating hormone must be present and being released.

One other point emerges from an examination of the tables, namely that temperature affects the ovulatory reaction, for at 20°C., egg-laying occurred earlier, *i. e.,* after a smaller number of grafted pituitaries, than at 14°C. This is in agreement with the findings of Bellerby and Rugh.[9]

8919 C

Precipitation and Color Reaction for Ascorbic Acid: Specificity of Acidified Sodium Selenite Solution.

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Certain compounds of selenium, notably selenious acid and its soluble salts, undergo reduction with the formation of free selenium, which appears as a brick-red precipitate or a brick-red or orange-colored colloidal solution. The smaller the quantity of reducing agent, the more the likelihood of the liberated selenium being sufficiently dispersed to form the colloidal state.

The organic compounds of biological significance which display reducing properties are aldehydes, ketones, carbohydrates with a free carbonyl group, polyphenols, thio compounds, including cysteine and glutathione, and ascorbic acid. The thio compounds reduce more readily than carbohydrates with a free carbonyl group, and ascorbic acid may reduce even more readily than thio com-

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[9] See footnote 6.

pounds. Ascorbic acid reduces even in the cold Fehling solution, alkaline or neutral solutions of silver salts, and potassium permanganate.[1]

To test the reducing action of ascorbic acid we have made a number of experiments with various compounds of selenium (selenic acid, sodium selenate, selenious acid and sodium selenite).

Ascorbic acid does not reduce in the cold or after heating on the water-bath either a 5% solution of selenic acid of sp. gr. 1.4 or crystalline sodium selenate solution (2%). When, however, the selenic acid is acidified with hydrochloric acid, reduction by ascorbic acid takes place gradually after heating or after prolonged standing. An acidified solution of sodium selenate (100 cc. of 2% sodium selenate plus 20 cc. concentrated hydrochloric acid) is also reduced by ascorbic acid. Hydrochloric acid reduces the selenate ion (SeO_4^{--}) in selenic acid and in sodium selenate to the selenite ion (SeO_3^{--}). The selenite ion in the form of selenious acid or sodium selenite is easily reduced to free selenium by a variety of organic compounds.

Certain thio compounds reduce selenic acid directly. Thiacetic acid reduces selenic acid (5% solution of selenic acid, sp. gr. 1.4) in the cold but cysteine, thioglycollic acid and thiobarbituric acid on the application of heat. Thiosalicylic acid shows no reducing action.

We find that ascorbic acid reduces in the cold selenious acid (5% solution of selenium dioxide). Emmerie has made a similar observation.[2] We also find that thioglycollic acid, cysteine and glutathione reduce this reagent at room temperature, while thioacetic acid, thiobarbituric acid and thiosalicylic acid cause reduction of the selenious acid reagent only after heating. The 2 compounds lacking a free thio or sulphydryl group, cystine and methionine, reduce neither at room temperature nor on heating.

Ascorbic acid reduces sodium selenite to elemental selenium. To test the effect of ascorbic acid on sodium selenite 3 reagents were employed: Reagent A—2% sodium selenite solution; Reagent B—2% sodium selenite in 10% sodium carbonate solution; Reagent C—100 cc. of 2%. sodium selenite solution plus 20 cc. concentrated hydrochloric acid.

We have reported in 1934 that ascorbic acid reduces all the 3 selenite reagents.[3] The thio compounds—ethyl mercaptan, o-thio-

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[1] Szent-Györgi, A., *Biochem. J.*, 1928, **22**, 1387.

[2] Emmerie, A., *Acta Brevia Neerland. Physiol. Pharm. Microbiol.*, 1934, **4**, 141.

[3] Levine, V. E., and Rosenthal, B. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1092.

cresol, α- and β-thionaphthol, thiosemicarbazide, thioacetic acid, thioglycollic acid, thiobarbituric acid, thiosalicylic acid, cysteine, and glutathione—also reduce the 3 selenite reagents. Ascorbic acid, however, reduces in the cold only the acidified selenite reagent (C), while the thio compounds require heat in order to reduce the same reagent. The thio compounds as well as ascorbic acid reduce in the cold the straight selenite reagent (A) and the alkalinized reagent (B).

Reduction of the selenite reagents is conveniently carried out by the interaction of 3 cc. of the reagent with 1 cc. of ascorbic acid solution or solution of thio compound. Due to the ease with which copper catalyzes the oxidation of ascorbic acid,[4] we have made up reagents as well as solutions of ascorbic acid with ordinary distilled water redistilled in glass. The water was thus freed from copper and iron. Copper was detected by McFarlane's method[5] using sodium diethyldithiocarbamate as the reagent described by Callan and Henderson.[6] The golden yellow color obtained in the presence of copper is extracted with amyl alcohol. Iron was detected by means of potassium thiocyanate, and the red color resulting is also extracted with amyl alcohol.

Ascorbic acid also reduces a solution of sodium tellurite to elemental tellurium, yielding a brownish black precipitate or a dark brown colloidal solution. The 3 reagents (A) 2% sodium tellurite solution; (B) 2% sodium tellurite in 10% sodium carbonate solution; (C) 100 cc. of 2% sodium tellurite solution plus 20 cc. concentrated hydrochloric acid are reduced in the cold by ascorbic acid. The alkalinized tellurite reagent is reduced by carbohydrates with a free carbonyl group. The 3 tellurite reagents are all reduced on heating by the thio compounds, o-thiocresol, ethyl mercaptan, thioacetic acid, thioglycollic acid, glutathione and cysteine. The acidified tellurite reagent is not as specific for ascorbic acid as the corresponding selenite reagent. Ethyl mercaptan and o-thiocresol reduce the acidified tellurite reagent in the cold, although thioacetic acid, thioglycollic acid, cysteine, and glutathione reduce this reagent only on heating.

The limit of sensitivity for ascorbic acid with reference to reduction in the cold of the acidified selenite reagent (C) is 0.04 mg. The limit of sensitivity with relation to the reduction on heating of the acidified selenite reagent is 0.01 mg. for o-thiocresol 0.025 mg.

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[4] Mawson, C. O., *Biochem. J.*, 1933, **29**, 569.

[5] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[6] Callan, T., and Henderson, J. A. R., *Analyst*, 1929, **54**, 650.

for ethyl mercaptan, 0.015 mg. for thioacetic acid, 0.003 mg. for thioglycollic acid, 0.025 mg. for glutathione, and 0.05 mg. for cysteine.

Glucoreductones, made according to the method of Kertesz[7] and neutralized with dilute hydrochloric acid, reduce all the 3 selenite reagents, but only the alkalinized selenite reagent (B) is reduced in the cold. The straight selenite reagent (A) and the acidified selenite reagent (C) are reduced only after heating. The glucoreductones do not reduce the acidified selenite reagent in the cold, while ascorbic acid does.

Carbohydrates with a free carbonyl group reduce after heating the alkalinized selenite reagent (B).[8] They also reduce the straight selenite reagent (A), but not as vigorously or profusely. Acid depresses the reducing activity of the carbohydrates with a free carbonyl group. The carbohydrates that reduce sodium selenite solution or the alkalinized sodium selenite solution on heating are arabinose, rhamnose, xylose, glucose, glucosamine, fructose, galactose, mannose, melibiose, cellobiose, lactose and maltose. Other carbohydrates, sucrose, trehalose, raffinose, melezitose, cellulose, starch, dextrin, glycogen, inulin, glucosides, glycoproteins and glycolipins, reduce only after hydrolysis and subsequent neutralization.

Compounds other than reducing carbohydrates and thio compounds do not reduce the alkalinized solution of sodium selenite, but reduce the acidified solution. This reduction is accomplished only after heating. Among the compounds reacting with the acidified selenite reagent (C) on heating, we may mention formaldehyde, paraldehyde, acetyldehyde, acetone, ethyl acetoacetate, β-hydroxybutyric acid, lactic acid, pyruvic acid, malonic acid, mucic acid, pyrocatchin, resorcin, pyrogallol, hydroquinone, phloroglucin, adrenalin, homogentisic acid, and creatinine. Orcein reduces but not as profusely as resorcin. Alkaline solutions are not desirable for testing the reducing property of polyphenols with respect to sodium selenite, since a red, red brown or dark brown coloration develops as a result of the hastened oxidation in an alkaline medium.

Homogentisic acid reduces in the cold the straight sodium selenite reagent (A) and the alkalinized sodium selenite reagent (B). The alkaline reagent tends to darken the mixture containing homogentisic acid due to the rapid oxidation induced by the alkaline medium. Addition of sodium carbonate solution alone to a homogentisic acid

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[7] Kertesz, Z. I., *J. Biol. Chem.*, 1934, **104**, 483.

[8] Levine, V. E., *Biochem. Bull*, 1915, **4**, 217.

solution causes the formation of red brown to black brown coloration which begins near the surface (proximity to oxygen). Homogentisic acid reduces the acidified selenite solution (C) poorly, but only after heating. Homogentisic acid (1 cc. containing 1 mg.) and 2 cc. of acidified selenite solution allowed to stand at room temperature showed faint reduction only at the end of 48 hours. Allantoin and uric acid do not reduce any of the selenite reagents at room temperature or at higher temperatures.

A brick red coloration is imparted in the cold to plant and animal tissues containing ascorbic acid when they are sprinkled with the acidified selenite reagent (C). We have observed this formation of the brick red color with lemon pulp, orange pulp, banana pulp, adrenal gland and liver.

Conclusion. Ascorbic acid reduces in the cold selenious acid, a straight sodium selenite solution, or one alkalinized or acidified. Reducing carbohydrates reduce only the alkalinized solution on heating. Thio compounds, including cysteine and glutathione, also reduce at room temperature the straight sodium selenite solution and the alkalinized solution, but the acidified solution only on the application of heat. A number of aldehydes, ketones, polyphenols, and creatinine also reduce the acidified selenite solution, but only on heating. Ascorbic acid differs from all the organic substances we have thus far tested, since it possesses the unique and specific property of reducing the acidified selenite reagent in the cold. The acidified sodium selenite reagent applied to plant and animal tissue rich in ascorbic acid is easily reduced in the cold with the formation of a brick red color characteristic of free selenium.

8920 P

Suppression of Persisting Corpora Lutea in Hypophysectomized Rats.*

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Although a great deal of information is available concerning the nature of the stimulus which causes a corpus luteum to form, little

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