phragm affords poor protection of the ovary against drying.

The subcutaneous ovary may be examined daily with the microscope,—the binocular, the ultrapaque or a mono-objective type; in the latter case a microlamp, an illuminating needle or a thin spatule of plastic material is placed directly beneath it or by perforating the tissues under the ovary.

When, through an error in operating, the hind leg of the rabbit rubs against the capsule, the sciatic nerve is sectioned to avoid irritation of the ovary, thus paralyzing the leg.

Precautions must be taken against vascular block,—as this might cause gangrene,—and against inflammatory irritations or damage to the nervous fibers. Obviously, this is not a subcutaneous graft of the ovary; it is a careful and adequate eventration, which maintains circulation and innervation intact.

With the technique for the biomicroscopic examination of the ovary and the Fallopian tube presented above, it is possible to follow, under the microscope, the development of the Graafian follicle, its circulatory changes, the exact moment when the "stigma" appears, its dehiscence, the flow of the follicular liquid carrying away with it the oöcyte, and the peristalsis of the Fallopian tube.

Observations already made are being prepared for publication.

Summary. For the microscopic observation *in vivo* of the mammal ovary and the Fallopian tube, two techniques are described, with special reference to the rabbit: (1) the transference of the ovary to a subcutaneous loggia, (2) the eventration of the ovary and its protection by a visualization capsule through which microscopic examination is possible. In both cases nervous and vascular connections are kept intact.

16336 P

Effect of Sodium Bicarbonate and Ammonium Chloride on Ascorbic Acid Metabolism of Adults.

BETTY E. HAWTHORNE* AND CLARA A. STORVICK.* (Introduced by J. S. Butts.)

From the School of Home Economics, University of Washington, Seattle, Washington.

A consistent lowering of the urinary ascorbic acid excretion of adults has been observed when an increased urinary pH is effected by the ingestion of sodium bicarbonate.[1,2,3] Guinea pig experiments measuring "scurvy scores" and body stores of ascorbic acid[4] indicated that the lowered excretion of ascorbic acid as a result of the ingestion of sodium bicarbonate appeared to represent an increase in retention rather than greater destruction as might be supposed from *in vitro* experiments. This communication reports for the first time, as far as we know, the results of simultaneous daily determinations of plasma and urinary ascorbic acid concentrations of adults on controlled ascorbic acid intakes ingesting sodium bicarbonate or ammonium chloride.

Experimental. The subjects were 2 adult women, C. C. and J. G., in normal health, both actively interested in nutrition research. The experiment was divided into 3 periods of 14 days each: 1st period—basal diet[5] (containing by analysis 7 mg of ascorbic

* Present address, School of Home Economics, Oregon State College, Corvallis, Oregon.

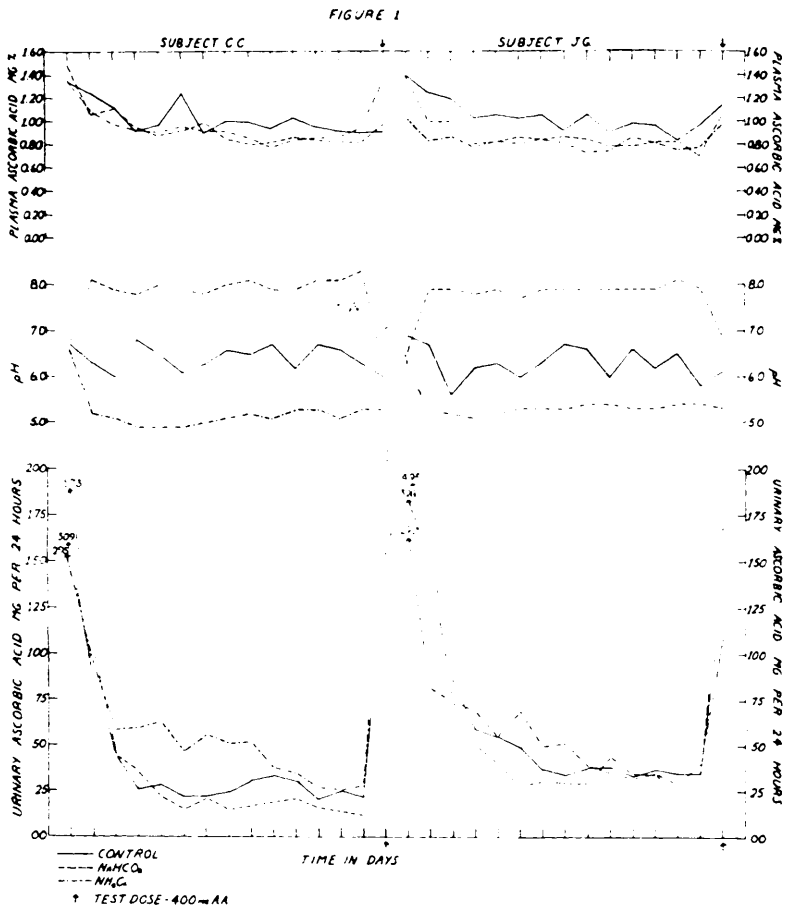
1 Heinemann, M., *J. Clin. Invest.*, 1941, **19**, 39.

2 Hawley, E. E., Stephens, D. G., and Anderson, G., *J. Nutrition*, 1936, **11**, 135.

3 Patterson, I., unpublished data, Cornell University, Ithaca, N.Y., 1942.

4 Hawley, E. E., Daggs, R. G., and Stephens, D. J., *J. Nutrition*, 1937, **14**, 1.

5 Lewis, J. S., Storvick, C. A., and Hauck, H. M., *J. Nutrition*, 1943, **25**, 185.



THE EFFECT OF SUPPLEMENTS OF AMMONIUM CHLORIDE AND SODIUM BICARBONATE ON THE URINARY pH, URINARY EXCRETION OF ASCORBIC ACID AND PLASMA ASCORBIC ACID CONTENT OF TWO HUMAN SUBJECTS

FIG. 1.

acid) plus 60 mg of crystalline ascorbic acid daily; 2nd period—basal diet plus 60 mg of ascorbic acid plus 15 g $NaHCO_3$ daily; and 3rd period—basal diet plus 60 mg of ascorbic acid plus 4 g NH_4Cl daily. Preceding each experimental period the subjects were saturated with ascorbic acid[6] and each period was concluded with a determination of response to a 400 mg test dose. The 60 mg supplement of ascorbic acid was dissolved in water and taken just before breakfast; the 15 g of sodium bicarbonate were given as 5 g portions dissolved in water at 10 a.m., 3 p.m. and 8 p.m.; and the 4 g of ammonium chloride were given as 2 g portions dissolved in water at 10 a.m. and 3 p.m. The pH and ascorbic acid determinations were made on 24-hour urine collections according to the method described by Belser, Hauck and Storvick.[6†] Fasting plasma ascorbic acid values were determined by the method of Farmer and Abt.[7]

Results. The results are illustrated in Fig. 1. When compared with the control period, sodium bicarbonate decreased and

[6] Belser, W. B., Hauck, H. M., and Storvick, C. A., *J. Nutrition*, 1939, **17**, 513.

† The authors wish to acknowledge the assistance of Catherine Cobb and Josephine Graham for these determinations.

ammonium chloride increased the urinary excretion of ascorbic acid. The mean daily urinary ascorbic acid excretions for the 3 periods are: C. C. 1st period, 26±1.3 mg, 2nd period, 19±2.0 mg, and 3rd period, 44±4.1 mg; and J. G. 1st period, 41±2.7 mg, 2nd period, 34±7.6 mg, and 3rd period, 46±4.3 mg. The differences between the periods were significant for C. C. but not J. G. tested according to Livermore's formula: differences must be 2 times the standard deviation of the differences.[8] The mean daily plasma ascorbic acid levels were consistently and significantly[8] decreased from the levels of the regular control diet with the addition of sodium bicarbonate or ammonium chloride. The mean daily plasma ascorbic acid values for the 3 periods are: C. C. 1st period, 0.98±.03 mg %, 2nd period, 0.87±.02 mg %, 3rd period, 0.89±.02 mg %; and J. G. 1st period, 0.98±.02 mg %, 2nd period, 0.81±.04 mg %, and 3rd period, 0.79±.01 mg %.

Hathaway and Meyer[9] have defined "utilization" as the difference between intake and excretion. By this definition the subjects' apparent "utilization" on the 3 periods was as follows: C. C., 61%, 72%, and 34%, and J. G., 39%, 49%, and 31%, respectively. Apparent "utilization" is then increased with the administration of sodium bicarbonate and decreased with ammonium chloride ingestion. However, the lowered mean plasma ascorbic acid values and the varied plasma and urinary excretion responses to the administration of a test dose at the completion of each period (Fig. 1) are not consistent with the apparent percentage "utilization."

Summary. This study indicates that the changes in the excretion of ascorbic acid are not accurate indications of utilization. The ingestion of both salts significantly lowered the mean plasma ascorbic acid content of the subjects indicating an interference with normal utilization. The reduced excretion of ascorbic acid with the ingestion of sodium bicarbonate both daily and in response to a test dose would seem therefore to represent more accurately increased destruction of ascorbic acid in the excretory process; and conversely, the increased excretion with ammonium chloride would seem to represent increased preservation in the excretory process.

[7] Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 1625.

[8] Hauck, H. M., personal communication, 1943.

[9] Hathaway, M. L., and Meyer, F., *J. Nutrition*, 1941, **21**, 503.

16337

Application of a Metabolic Inhibitor to the Developing Chick Embryo.

W. WILBUR ACKERMANN* AND ALFRED TAYLOR.

From the Biochemical Institute, The University of Texas, and the Clayton Foundation for Research, Austin.

This research is the first of a series of attempts to apply a new method to the study of chemical embryology. The usefulness of metabolic analogs (anti-vitamins, anti-metabolites) in the study of the metabolism of microorganisms has been fully demonstrated by Shive and his co-workers in this laboratory by the development of the method of "inhibition analysis".[1,2] An outgrowth of these findings, which has been in prospect in this laboratory for some time, is an attempt to block metabolic reactions in embryonated and cancer-bearing eggs and to apply the same methods of study to these tissues. A study of the effect of precursors of the competing metab-

* Rosalie B. Hite Fellow.

[1] Shive, W., and Macow, J., *J. Biol. Chem.*, 1946, **162**, 451.

[2] Beerstecher, E., and Shive, W., *J. Biol. Chem.*, 1946, **164**, 53.