consecutive cycles, the shapes of the excretion curves were similar in both cycles. In four cycles, a brief, but significant, fall in excretion occurred midmenstrually and lasted for one or two days. In four cycles, the higher level of citric acid excretion persisted during most of the second half of the cycle, to fall abruptly two or three days before the next flow. The maximal midmenstrual increases in citric acid excretion over the menstrual levels were 225, 240, 275, 350, 450 and 500 mgs for the six cycles studied.

The accompanying graph (Fig. 1) illustrates the

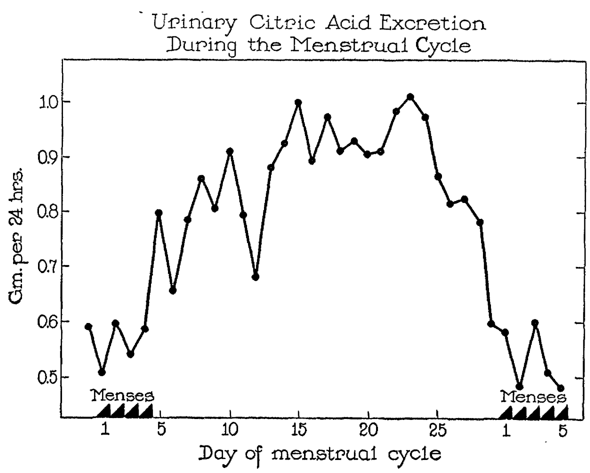

FIG. 1.

characteristics of the curve of citric acid excretion during the menstrual cycle of one of the subjects. The post-menstrual rise, the sharp midmenstrual dip, the persistence of the high level of excretion during most of the premenstrum, and the abrupt fall to the previous menstrual levels, were particularly striking in this subject.

Results, of a preliminary character, can be reported of experiments designed to analyze the relation of the individual steroidal reproductive hormones to the cyclic alterations in citric acid excretion during the menstrual cycle. An estrogenic hormone, (estradiol benzoate), was administered to two amenorrheic girls in amounts that did not exceed their estrous requirements, as judged by vaginal smears. Both subjects showed significant increases in citric acid excretion (230 and 500 mgs, respectively), during its administration, and a sharp return to lower levels on cessation of treatment. We are now investigating the possible influence of progesterone on citric acid excretion, in view of the fact that the high level of excretion is generally maintained throughout most of the second half of the cycle. The reverse effect on citric acid excretion was observed when an androgen, testosterone, was administered to a male with pituitary hypogonadism. During two courses of treatment, urinary citric acid fell significantly, (300 mgs/24 hrs.), below the control values and rose sharply to previous levels when treatment was terminated. In one amenorrheic girl, testosterone propionate likewise reduced the daily output of citric acid by 175 mgs. Additional studies on human subjects and animals are under way to ascertain the constancy and prevalence of these phenomena.

The studies on the female subjects were correlated with vaginal smears. The results of the correlation of this index of ovarian function with the excretion of citric acid will be reserved for a subsequent report.

These observations would appear to establish the existence of a cycle of citric acid excretion which bears a definite relation to the menstrual cycle and is probably hormonally conditioned. This cycle may result from a direct effect of the hormones involved on citric acid metabolism, or from their influence on some other mechanism, such as acid-base regulation, renal function or carbohydrate metabolism, which in turn influences citric acid formation and excretion. Since citric acid is but one of the organic acids eliminated in the urine, it will be necessary to determine whether or not excretion of other organic acids is also altered under these conditions, before the changes in citric acid excretion can be regarded as specific. The bearing of these results on all previous experiments dealing with citric acid excretion in women is obvious.

Although these experiments throw no new light on the basic problem of the endogenous metabolism of citric acid, they do point out a new functional relationship, and should provide a valuable tool to aid in its study.

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PHYSIOLOGIC STUDIES ON THE CORNEA

THE cornea is a composite membrane forming the anterior portion of the outer coat of the eye-ball. In all vertebrates it has on its anterior surface a thin, stratified epithelial layer and on its posterior surface a single layer of endothelial cells. Just anterior to the endothelium is a homogeneous elastic lamina (Descemet's membrane), and just beneath the epithelium there is, in some species, a homogeneous glass-like lamina (Bowman's membrane). The remainder of the cornea, the substantia propria, comprising about 90 per cent. of its total thickness, is made up of collagenous fibers and supporting cells arranged parallel to the surfaces of the cornea. These fibers merge into those of the sclera without any abrupt histologic transition corresponding to the change from the transparent to the opaque portion of the corneo-scleral coat.

The permeability, the degree of hydration, the transparency and the absence of blood vessels are four properties which distinguish the cornea from the sclera, and indeed from most other tissues. These properties have been extensively studied in this laboratory during the past two years and, as a result of the new findings, especially as regards permeability, have led to a new concept of corneal physiology. The data are to be reported in detail elsewhere.[1] A preliminary report of some of the findings and conclusions which are of general interest are presented here.

The excised cornea was found to be, in effect, impermeable in either direction to NaCl but freely permeable in both directions to H_2O. Removal of the epithelium caused the cornea to become permeable to NaCl, from which it was concluded that the epithelium was an effective semipermeable membrane with respect to NaCl. The permeability of the endothelium could not be adequately studied with the excised cornea because of damage resulting from the manipulation. But if the corneal epithelium is first removed the properties of the endothelium may be studied in the intact eye. In this manner it was shown that the endothelium is substantially like the epithelium in being an effective semipermeable membrane with respect to NaCl. Thus the substantia propria, while permeable to NaCl and water, is bounded on either side by membranes permeable only to water.

The normal degree of corneal hydration appears to be quite unusual, for when pieces of cornea are immersed in various aqueous solutions they swell several hundred per cent. This was found to be true, even though the bath fluid was blood serum or aqueous humor and despite wide variations in hydrogen ion concentration or in tonicity of the solutions. The only way in which the explanted cornea could be maintained in a state of dehydration comparable to that existing normally was by maintaining an osmotic gradient across the intact epithelium or endothelium, or both, so that water was abstracted from the cornea as rapidly as it became available in the stroma. Presumably a similar mechanism is operative *in vivo;* fluid diffuses into the cornea from the vascular plexus at the corneo-scleral junction, and its water is continuously transferred into the hypertonic tears on the anterior surface and into the hypertonic aqueous on the posterior surface.

The maintenance of corneal dehydration is essential for transparency. Removal of the semipermeable membranes or lowering the tonicity of the bath fluids will allow the cornea to swell and become opaque. Presumably the interstitial fluid, having a different refractive index from that of the structural components of the cornea, must be removed or prevented from accumulating in order to maintain optical homogeneity.

Correlated with the dehydrating mechanism as described above is the effect on circulation. The cornea has no vascular or lymphatic vessels, and nutritive material is provided by diffusion from the periphery. It now seems likely that, in addition to diffusion, the continuous abstraction of water from the corneal surfaces ensures a movement of fluid which serves to transport oxygen and other dissolved materials from the blood to the corneal tissues.

By contrast the sclera has no semipermeable membranes and therefore no dehydrating mechanism as described above for the cornea. In consequence, the sclera is normally hydrated to its physiologic limit and is opaque. If, however, it is artificially dehydrated, as by drying in air, it becomes transparent like the cornea. The optical difference, then, between the cornea and sclera appears to be due, not to any structural difference in their respective fibers, but to the fact that the former has a dehydrating mechanism not present in the latter.

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[1] *Archives of Ophthalmology.*

PHYSIOLOGICAL ACTIVITY OF ASCORBIC ACID IN PLANT LIFE

The diurnal variation of ascorbic acid in tomato plants previously noted,[1] if common to all plants, merits further consideration in determining the best time to harvest edible greens. If greens are to be shipped or canned, is it preferable to cut them in the evening or in the morning? Using young tomato plants grown in pots as subject material, a number of pertinent experiments have been made.

The plants to be allowed to grow overnight were washed free of soil gently to avoid root injury; adhering water was blotted off with absorbent paper; the plants were weighed and at once replanted. Such repotted plants gained very little in weight overnight even though the pots were standing in water to cover the soil, whereas cut plants standing in water always gained appreciably.

Knowing the change in weights of the plants held overnight, both cut and growing, it was possible to calculate the ascorbic acid content in the morning back to the weight of the previous evening and thus eliminate the dilution effect. Typical results are given

[1] Science, December 13, 1940, p. 561.