SPECIAL ARTICLES

COPPER AND VITAMIN C EFFECTS UPON THE EXCISED FROG HEART[1]

IN a preliminary report, Urban and Peugnet[2] showed that vitamin C exerts a powerful beat-strengthening action upon the perfused frog heart. Since that time, Peugnet and Urban[3] have shown that: (1) Vitamin C also accelerates the beat frequency of the abnormally slow pacemaker, with little or no effect upon the normal rate—in which it differs from adrenalin; (2) vitamin C frequently increases the diastolic "tonus," though not as yet reproducibly; (3) inotropic effects are obtainable in concentrations as low as .05 mg per cent.; (4) vitamin C action is independent of and additive with that of perfusate glucose; (5) vitamin C action is not due to atropine-like vagus paralysis, nor to removal of inhibitory action of perfusate potassium, nor to calcium contamination of the vitamin C; (6) oxidized (iodine) vitamin C is inactive.

In the present communication, the author wishes to present experiments proving that the action of vitamin C on the frog heart requires the presence of copper.

The method is that of Straub, described in detail by McLean and Hastings.[4] Ringer (glucose-free) solutions are made with Kahlbaum (analytical) chemicals and Pyrex-distilled water, and contain $NaHCO_3$ (0.2 per cent.), being continuously bubbled, both in reservoir vessels and in cannula, with 95 per cent. O_2–5 per cent. CO_2. Vitamin C is the synthetic crystalline material.[5]

The experimental evidence for the necessity of copper in the action of vitamin C is briefly as follows:

(1) The inotropic action is always present (about 40 experiments) with ordinary "reagent quality" chemicals and metal-distilled water.

(2) The effect is absent (14 out of 16 experiments) with Kahlbaum chemicals and Pyrex-distilled water.

(3) The heart which fails to respond to vitamin C alone does respond when copper is added to the perfusate (0.1–4.0 micro-mols per liter copper ion, as chloride).

(4) In the same heart, copper alone is ineffective until the addition of vitamin C.

(5) Vitamin C alone can not maintain the inotropic increase, but requires the continued presence of copper in the perfusate.

(6) The heart which does respond to vitamin C alone gives a greater response with copper in addition.

It is particularly to be noted that the response to the addition of copper (vitamin C already present) occurs only after a latent period, or "induction" period, whereas the response to vitamin C addition (copper already present) begins immediately. (The technique in the two cases is as nearly identical as possible; the copper and the vitamin C are freshly mixed with Ringer a few seconds before each application.) This induction period is never seen with vitamin C, and appears to be a constant phenomenon with copper. The duration of the latency varies roughly inversely with the copper concentration (see Table I).

TABLE I

Frog Date	Copper Ion. micro-mol./L	Induction per. (sec.)
5–25–39	0.4	240.
6–4	2.0	90.
5–10	2.5	110.
5–24, A	4.0	30.
5–24, B	4.0	35.

As to the nature of the induction period, it appears certain that this delay is not due to any *in vitro* reaction or "activation" time, since the time during which the two are in contact *in vitro* is the same whether or not the tissue has been previously exposed to the copper alone; and, as already mentioned, the previous "conditioning" of the tissue with copper abolishes the delay. Apparently, then, the copper is necessary in some part of the tissue system which utilizes vitamin C. Further experiments on this question will be presented at a later date.

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[1] Aided by grant of the Ella Sachs Plotz Foundation.

[2] F. Urban and H. B. Peugnet, *Am. Jour. Physiol.*, 123: 207, 1938.

[3] Exhibited with F. Urban at the American Medical Association Convention, May, 1939.

[4] F. C. McLean and A. B. Hastings, *Jour. Biol. Chem.*, 107: 337, 1934.

[5] Donated by Merck and Company.

PROLONGED THERAPEUTIC EFFECT OF SUBCUTANEOUSLY IMPLANTED CRYSTALS OF OVARIAN HORMONE IN WOMEN

THE investigation reported here was undertaken to determine whether it is possible to prolong the physiologic effects of a given dose of estrogenic hormone in humans by implanting crystals of the hormone, subcutaneously. It appears from the results of various investigations that the high dosage of estrogens required to relieve symptoms caused by ovarian failure and to maintain the endometrial and vaginal mucosa in a normal physiologic state is in a great part attributable to the rapid absorption and excretion of the hormone. To achieve a satisfactory therapeutic effect at present it is necessary to administer intramuscular