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

injections of a solution of the hormone in oil at frequent intervals for periods of many weeks. Aside from the inconvenience caused the patient by the necessity for frequent injections, there is the additional factor of expense which places this form of therapy beyond the means of the majority of patients. In the interests of economy and the patient's comfort, it would appear to be highly advantageous to have an estrogenic preparation which would be absorbed slowly so that the patient may derive a fuller measure of benefit from a given amount of the hormone and obviate the necessity for frequent injections. An attempt was made to achieve this objective by the implantation of crystalline a-estradiol benzoate, subcutaneously.

TECHNIQUE

Crystals of the hormone were placed in small glass tubes measuring 3 mm in diameter and 1½ cm in length and sterilized by autoclaving. The amounts varied from 4 to 7 mgm. The implantation was performed in the gluteal region. The skin was prepared with tincture of iodine and alcohol. An area of skin measuring approximately 1½ inches in diameter was infiltrated with 2 per cent. novocaine. An incision measuring 1 inch in length was made and the capillary oozing controlled with a dry packing. The estradiol benzoate crystals were then implanted into the wound and the skin edges approximated with 2 silk sutures. The sutures were removed 5 days later.

A total of 10 menopause cases were so treated, including patients with surgical as well as natural menopause. All the patients had typical menopause symptoms and morphologic evidence of estrogen deficiency. The vaginal smear was used as an indicator of estrogen deficiency and estrogenic effect. Vaginal smears were taken twice weekly after the implantation.

RESULTS

The majority of the patients reported improvement in their symptoms as early as 6 days after the implantation. Complete relief of symptoms usually occurred within 2 weeks and has persisted to date (periods varying from 60 to 98 days after the implantation). Objective evidence of the biologic effect of the implanted crystals was noted in the vaginal smear changes as early as 4 days after the implantation. The "atrophy" cells and leucocytes began to diminish in number, being replaced by large squamous epithelial cells. At the end of 7 to 10 days, the smears consisted entirely of large squamous epithelial cells exhibiting the characteristics of the smear of the normally menstruating woman. This evidence of continued activity has persisted to date (periods varying from 60 to 98 days).

Inasmuch as 1 mgm of a-estradiol benzoate is equivalent to 6,000 R.U., these patients were given doses varying from 24,000 to 42,000 R.U. of estrogenic hormone. This amount, when administered in solution in oil, in a single dose, intramuscularly, will, in the average menopause case with a well-developed menopause syndrome, produce only an incomplete effect both on the symptoms and vaginal smears. Unless repeated injections are administered the symptoms recur in their original intensity and the smears show evidence of regression to the pre-treatment state within 7 to 14 days after the injection.

It appears that crystalline a-estradiol benzoate, when implanted subcutaneously in women who have clinical and morphologic evidence of ovarian deficiency, exerts an effect which is strikingly more pronounced and more prolonged than is obtained with comparable doses of the hormone administered intramuscularly in solution in oil. This prolonged effect is attributed to the slow rate of absorption and excretion of the hormone.

Judging from the prolonged effect exerted by the small amounts of hormone used here, it is logical to expect that by implanting larger amounts of hormone (25 to 50 mgm) it will be possible to keep a patient symptom-free for periods of many months. This method of administering estrogens, it seems to us, has great therapeutic potentialities. In addition to being applicable to various types of cases of ovarian failure (natural menopause, functional amenorrhea, x-ray and radium castrates, etc.), it is suggested that it can be employed prophylactically at the time of surgical removal of the ovaries to prevent the development of the artificial menopause.

Further studies are being carried on in a larger series of cases to determine the duration of the effect in relationship to different amounts of the hormone implanted in order to ascertain the optimal amount of hormone for various clinical conditions.[1]

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THE INFLUENCE OF 3-INDOLE-ACETIC ACID ON POLLEN GERMINATION

THE presence of auxin in various kinds of pollen has been shown by several investigators.[1] Although several hundred papers have been published on growth substances, apparently no work has been done on the influence of auxin on the germination and growth of pollen tubes. The present writer is studying the effects

[1] We are indebted to Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J., for the crystalline a-estradiol benzoate used in this investigation.

[1] F. W. Went and K. V. Thimann, "Phytohormones." New York, 1937.