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.

of heteroauxin on the rate of germination, the percentage of germination and the rate of elongation of the pollen tubes of several different species. The results obtained from five species seem worthy of a preliminary report.

TABLE I

MEASUREMENTS ON POLLEN GERMINATION AND TUBE GROWTH WITH AND WITHOUT 3-INDOLE-ACETIC ACID

| Pollen | Rate of germination in minutes | | No grains counted | | Percentage germination | | Average four-hour tube length in microns* | |
|---|---|---|---|---|---|---|---|---|
| | Test | Control | Test | Control | Test | Control | Test | Control |
| *T. occidentalis* .... | 2–3 | 4–6 | 1453 | 1274 | 84% | 61 % | 920 | 590 |
| *T. canaliculata* .... | 2–3 | 4–6 | 1240 | 1042 | 80 " | 62.5 " | 350 | 208 |
| *Polygonatum* ..... | 15–18 | 25–28 | 1272 | 1240 | 58 " | 37 " | 450 | 220 |
| *Lathyrus odoratus* . | 25–30 | 90–100 | 1475 | 1535 | 76 " | 40 " | 163 | 86 |
| *Pinus Austriaca* ... | 6 hrs. | No ger. | 1200 | 1500 | 51 " | 00 " | 75 | 00 |

* The tube measurements on *Pinus* were made in twelve hours.

Pollen grains of *Tradescantia canaliculata, T. occidentalis, Polygonatum commutatum, Lathyrus odoratus* and *Pinus Austriaca* Höss were cultured in solutions of sucrose (C.P.) in twice-distilled water. A 3 per cent. sugar solution was used for *Polygonatum* and the two species of *Tradescantia*; a 10 per cent. solution was used for *Lathyrus* and *Pinus*. These concentrations were chosen because preliminary trials had shown that they caused no plasmolysis or plasmoptysis. For each experiment two solutions were prepared with exactly the same concentration of sucrose, and to one of them enough 3-indole-acetic acid was added to be of a concentration of one part per million parts solution. The other solution was used as a control. The solutions were made up each time a group of cultures were started in order to prevent bacteria and mold from bringing about changes. The pH of the solutions was $6.4 \pm 0.1$ as determined by the calomel electrode potentiometer. Brink's[2] hanging drop technique was employed. Although no attempt to control the temperature was made, it varied only within the range of 25°–27° C. A large number of slides were carried through for each species in both the sugar series and the sugar-heteroauxin series. Representative fields were counted from each slide by means of the compound microscope (100X). To determine the rate of germination, the elapsed time between inoculation and the first protuberance of the intine was noted on each slide. Extensive four-hour counts were made on all species except *Pinus* (in which case twelve-hour cultures were counted) to determine the percentage of germination. Measurements of the pollen tubes were made also on these four- and twelve-hour cultures using an ocular micrometer.

These data indicate that 3-indole-acetic acid in concentrations of 1:1,000,000 produces the following effects: (1) germination is stimulated in such a way that the pollen tube appears in considerably less time than in the control, (2) the rate of elongation of the tube is increased, (3) a much higher percentage of germination is achieved, (4) greater tube lengths are found at the end of four hours, and (5) the pollen of *Pinus Austriaca* is stimulated to germinate, whereas no germination occurred in the control.

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[2] R. A. Brink, *Am. Jour. Bot.*, 12: 149–162, 1925.

## THE VITAMIN C CONTENT OF SPRING GREENS

IN connection with a course in local flora, determinations were made of the ascorbic acid (vitamin C) content of certain wild plants which have been reported as being used for spring "greens" in southern Ohio. Young leaves, collected during the spring as soon as good growth had started, were analyzed in duplicate by the 2,6 dichlorophenolindophenol indicator method as described by Bessey.[1] The results are reported as milligrams of ascorbic acid per gram of fresh weight.

TABLE 1

ASCORBIC ACID CONTENT OF CERTAIN WILD PLANTS USED AS SPRING GREENS

| Common name | Botanical name | Mg ascorbic acid per gram of fresh weight |
|---|---|---|
| Burdock | *Arctium minus* | 0.696 |
| Chickweed | *Stellaria media* | 0.377 |
| Curly dock | *Rumex crispus* | 1.349 |
| Dandelion | *Taraxacum officinale* | 1.546 |
| Milkweed | *Asclepias syriaca* | 6.556 |
| Pokeweed | *Phytolacca americana* | 2.735 |
| *Shepherd's purse | *Bursa bursa-pastoris* | 1.296 |
| Skunk cabbage | *Spathyema foetida* | 3.150 |
| *Slender nettle | *Urtica gracilis* | 1.007 |
| Smaller leaved milkweed | *Asclepias incarnata* | 2.537 |
| Sorrel | *Oxalis stricta* | 1.765 |
| Sow thistle | *Sonchus oleraceaus* | 0.633 |
| *Water cress | *Sisymbrium nasturtium-aquaticum* | 1.875 |
| *Wild carrot | *Daucus carota* | 0.748 |
| Wild lettuce | *Lactuca canadensis* | 0.636 |

[1] Otto A. Bessey, *Jour. Am. Med. Asn.*, 111: 1290–98, 1938.