the injected animals, higher doses were not administered.

Lipo Adrenal Cortex was found to inhibit the gonadotrophic hormone secretion at 1 rat unit per day and to produce uterine stimulation at 2 rat units per day. The gonadotrophin inhibiting activity of 1 rat unit of Lipo Adrenal Cortex can be estimated as equivalent to approximately 0.01 γ of alpha-estradiol. Adrenal Cortex Extract produced partial inhibition of gonadotrophin secretion in daily doses of 4 rat units. Due to the large amount of fluid required, higher doses were not tried. We can conclude that Adrenal Cortex Extract has less than one-fourth the gonadotrophin inhibiting activity of the Lipo Adrenal Cortex. The gonadotrophic inhibiting activity of the Lipo Adrenal Cortex and the Adrenal Cortex Extract is not proportional to their adrenal cortical activity, and is probably due to estrogenic substances obtained from the adrenals in the extraction procedures. To the best of our knowledge, DCA has not been isolated from these adrenal preparations, and does not account for the gonadotrophin inhibiting activity of the Lipo Adrenal Cortex or the Adrenal Cortex Extract.

The possibility of interference with the pituitary-ovarian interrelationship should be considered in the administration of these hormones in experimental and therapeutic procedures.

Summary. Lipo Adrenal Cortex and desoxycorticosterone acetate were found to stimulate uterine growth and to inhibit pituitary gonadotrophic hormone secretion when administered to female rats in relatively small amounts. Adrenal Cortex Extract partially inhibited gonadotrophin secretion but was less than one fourth as effective as the Lipo Adrenal Cortex. Large doses of Compound E failed to inhibit the gonadotrophin secretion. The secretion of gonadotrophic hormone is inhibited in *immature rats* by steroids in amounts less than those which result in uterine growth.

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Effect of Folic Acid, Aminopterin and Vitamin K on Growth of Roots of *Allium cepa.** (17887)

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Previous reports have described the effect of colchicine on nuclear divisions in onion root tips(1) and in human tumors(2). The action of colchicine, acenaphthene and a combination of colchicine and x-ray treatments have also been reported(3). It seemed of interest to study the effect of folic acid (pteroylglutamic acid) and its antagonist aminopterin (4-amino pteroylglutamic acid) to determine the influence these agents have on the rate of growth of fundamental tissue since they have been used as theraputic agents in the treatment of cancer. Vitamin K substitute (2-methyl 1,4-naphtho-hydroquinone-diphosphoric acid ester, tetra sodium salt plus 6 H_2O) another agent that gained some popularity in the treatment of neoplasia was studied and compared with colchicine, folic acid and its antagonist in the light of recent claims that this substance combined with

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1. Levine, M., and Gelber, S., *Bull. Torrey Bot. Club*, 1943, v70, 175.

2. Levine, M., Silver, G. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 54.

3. Levine, M., *Bull. Torrey Bot. Club*, 1945-1946, v72, 563; v73, 34; v73, 167.

TABLE I.

Agent and conc., g/ml	Action on root elongation: No. of roots	Avg total growth 10 days, mm	% inhibition*	Avg mitotic frequency per 2000 cells: 1st 5 days	2nd 5 days	% reduction†: 1st 5 days	2nd 5 days
Controls	80	84.3	00.0	2.62	3.46	0.0	0.0
F.A. 10^{-5}	32	10.3	87.8	1.76	1.06	32.8	69.4
" 10^{-7}	56	31.5	62.6	2.54	1.52	3.1	56.1
" 10^{-9}	32	44.3	47.4	1.86	1.56	29.0	54.9
Amin. 10^{-7}	32	2.5	97.0	0.23	1.32	91.3	61.8
" 10^{-9}	32	38.7	54.1	2.16	1.58	17.6	54.3
" 10^{-10}	32	52.2	38.1	1.98	1.18	24.4	65.9
Vit. K subs.							
10^{-4}	32	6.2	92.6	1.56	2.06	40.5	40.5
10^{-5}	32	31.5	62.6	2.18	1.40	16.8	59.5
10^{-7}	32	52.8	37.4	2.34	1.54	10.7	55.5

* The % inhibition is the difference of the % of total growth from 100%. The % total growth is the fraction the treated roots grew as compared to the controls.

$$\% \text{ inhibition} = 100\% - \frac{\text{total growth of treated roots}}{\text{total growth of controls}} \times 100$$

† The % reduction is the difference of the % of mitosis in the treated roots from the controls divided by the % of mitosis in the controls.

$$\% \text{ reduction} = \frac{\% \text{ mitosis controls} - \% \text{ mitosis treated roots}}{\% \text{ mitosis controls}} \times 100$$

x-rays inhibited growth of tissue components grown *in vitro*(4).

Methods and materials. *Allium cepa* var. Yellow Globe, the root tissue of which is readily adapted to the root smear technic, was used in this study. The bulbs were selected on the basis of approximate uniformity of weight (60 to 80 g) freedom from bruises and infections. The onions were germinated in tap water in opaque cylindrical jars of 240 ml capacity for 3 days. Each experimental series was started with a larger number of bulbs than was actually treated, more than 200 onions were used. After the germination period, the selected onions were placed in water in which the agent under investigation was dissolved or suspended. Folic acid and aminopterin solutions were made from stock suspensions containing 20 mg/100 ml. Attempts to dissolve these materials by the addition of small quantities (1 to 5 ml) of 95% alcohol or ether failed. The vitamin K substitute (Hoffmann-LaRoche, Synkovite) was water soluble and the solutions were made from stock containing 100 mg/100 ml. Each of these substances was tested at an initial concentration of 10^{-7} g/ml. Linear growth of the root was measured at the time of immersion and thereafter daily for 10 days. Root tips removed for cytological examination were prepared by the root smear technic and stained with Sudan Black B as described by Cohen(5) with the omission of the propionic acid.

Observations. Folic acid at concentrations of 10^{-5}, 10^{-7} and 10^{-9} g/ml, aminopterin at concentrations of 10^{-7}, 10^{-9} and 10^{-10} g/ml, and vitamin K substitute at concentrations of 10^{-4}, 10^{-5} and 10^{-7} g/ml had an inhibitory effect upon the linear growth of onion roots. Folic Acid at the above concentrations had no other obvious toxic effect upon the roots. These roots were glistening white, turgid and appeared normal. At concentrations of 10^{-7} and 10^{-9} g/ml the increment in length after 1 day immersion was close to that of the controls, but thereafter the difference between the two became more pronounced. Folic acid at a concentration of 10^{-5} g/ml suppressed growth during the whole immersion period. The average total lengths of the treated roots and controls appear in Table I and Fig. 1. Aminopterin at a concentration of

4. Mitchell, J. S., and Simon-Reusse, I., *Nature*, 1947, v160, 98.

5. Cohen, I., *Stain Tech.*, 1949, v24, 177.

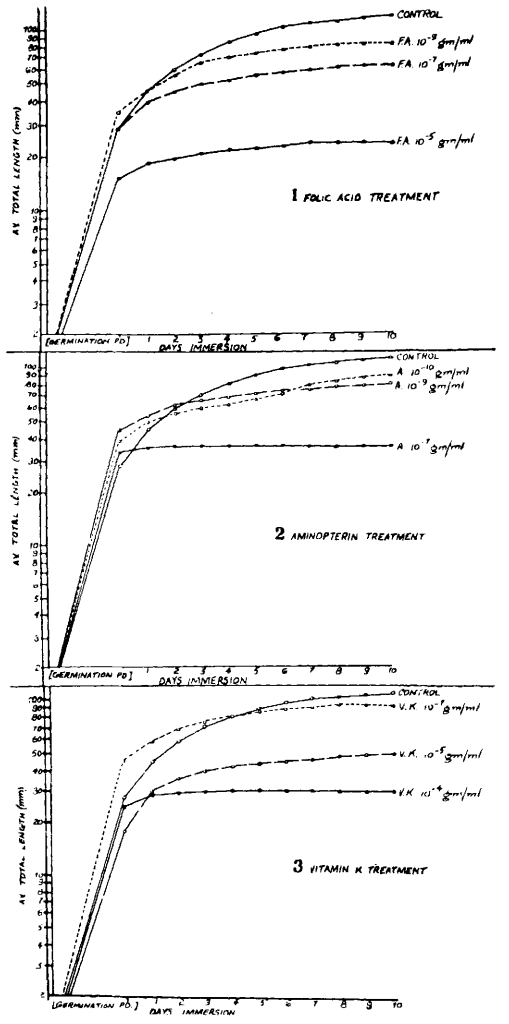

FIG. 1.

10^{-7} g/ml caused some of the roots to become flaccid and slightly yellowish in color after 5 days immersion. Throughout the experimental period aminopterin at this concentration depressed root growth and after 4 to 6 days immersion, root growth ceased. With concentrations of 10^{-9} and 10^{-10} g/ml the average daily increments in length were smaller than those of the controls. The total lengths of the treated roots and controls are shown in Fig. 2. Vit. K substitute at concentrations of 10^{-4}, 10^{-5} and 10^{-7} g/ml showed inhibitory effects on root growth. At a concentration of 10^{-4} g/ml the agent showed an effect similar to aminopterin at a concentration of 10^{-7} g/ml. After immersion of 5 to 6 days root growth ceased and some roots became flaccid and yellowish in color. Vit. K substitute at concentrations of 10^{-5} and 10^{-7} g/ml showed inhibitory effects of the same order of magnitude as folic acid at concentrations of 10^{-7} and 10^{-9} g/ml (Table I and Fig. 3). These studies showed that in every instance growth inhibition increased with the concentration of the agent (Table I and Fig. 1).

Microscopic studies of the treated and control roots were made by the smear technic together with fixed and paraffin sectioned material. The tissue was examined for karyokinetic aberrations and the cells counted under high dry magnification. The percentage of mitoses was based on counts of 2000 or more cells. In all but 2 instances there is a fall in the 5-day averages of the mitotic index as the immersion time lengthens (Table I). The variations in the percent of mitosis in the treated roots may represent inherent properties of the tissue as the controls manifest similar variations. The number of mitoses in the controls is consistent with the counts made on similar material by Levine and Gelber(1). No cellular abnormalities such as arrested metaphases or polyploidy were noticed in the treated roots. In general, the treated roots showed a smaller percent of mitosis than the controls. While the percent of division figures was depressed there were mitotic stages present in all of the roots after treatment. The inhibition of growth by these agents may be due to an effect upon the ability of the root to elongate and with some impairment of the mitotic process.

Discussion. In the studies described here folic acid at a concentration of 10^{-5} g/ml and aminopterin at a concentration of 10^{-7} g/ml had similar inhibitory effects on growth (Table I). The ratio of these concentrations, folic acid to aminopterin is 100:1, the same relative potencies observed when these compounds were used on frog oviducts(6). Mitchell and Simon-Reusse(4) studied the effect of vitamin K substitute in combination

6. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., *Cancer Res.*, 1949, v9, 605. Abst.

with x-rays on tissue cultures of chick fibroblasts. Vit. K substitute alone and in combination with x-rays, it was observed, produced mitotic inhibition. In general in our studies it was observed that the treated roots besides showing an inhibition in linear growth had a lower mitotic percentage than the controls though it must be emphasized that no karyokinetic abnormalities were observed.

Summary. The effect of folic acid, aminopterin and vitamin K substitute dissolved or suspended at given concentrations in water, on the growth and development of roots from bulbs of *Allium cepa* was investigated. Folic acid at concentrations of 10^{-5}, 10^{-7} and 10^{-9} g/ml, aminopterin at concentrations of 10^{-7}, 10^{-9} and 10^{-10} g/ml and vitamin K substitute at concentrations of 10^{-4}, 10^{-5} and 10^{-7} g/ml inhibited the linear growth of roots of *Allium cepa*. The percent of mitosis in the treated roots was in general lower than that of the controls. No abnormalities of the mitotic figures in the treated root tips were observed. The inhibition of growth by these agents may be due partially to an interference with the process of cellular elongation and differentiation following karyokinesis and partially by reducing the rate of nuclear division.

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Biuret Reaction as Applied to Determination of Antibody Nitrogen. (17888)

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The quantitative biuret reaction(1) has been successfully applied to the determination of protein in urine(2), blood serum(3) and cerebrospinal fluid(4). Since the method is simple and rapid, it appeared desirable to investigate its use in immunochemical procedures. Various proteins may yield biuret colors whose absorption maxima differ slightly in intensity and position(5). In a series of determinations of optical density per mg N at 540 mμ relative to bovine serum albumin, we obtain values of 0.95, 0.98, 1.08, and 1.12, respectively for horse, rabbit, rat and dog serum, 1.01 for crystalline egg albumin, and 0.98 for bovine γ-globulin. With antigen-antibody precipitates in which the components give similar absorption maxima, the error encountered should be negligible. Correlation with nitrogen content may be obtained by the use of proper standardization procedures.

Materials and methods. Antigens. Crystalline egg albumin and crystalline bovine albumin were used in these studies. For immunization procedures, the albumins were precipitated with alum; for antibody precipitation, they were dissolved in 0.85% NaCl solution. The nitrogen contents were determined by the micro-Kjeldahl method. *Antisera**. Albino rabbits weighing about 1.5 kg were given 4 series of 4 intravenous injections of antigen increasing from 1.5 mg to 7.5 mg. Rest periods of 3 days were allowed between series, and 7 days after the last injection blood was withdrawn aseptically. Sera were preserved by the addition of 0.01% sodium merthiolate. *Precipitation of antibody.* Antibody was precipitated according to the method of Heidelberger and Kendall(6) as described by Kabat and Mayer(7). *Biuret reaction.* The biuret reagent described by

1. Autenrieth, W., and Mink, F., *Münch. med. Wochschr.*, 1915, v62, 1417.
2. Hiller, A., PROC. SOC. EXP. BIOL. AND MED., 1928, v24, 385.
3. Robinson, H. W., and Hogden, C. G., *J. Biol. Chem.*, 1940, v135, 707.
4. Dittebrandt, M., *Am. J. Clin. Path.*, 1948, v18, 439.
5. Sizer, I. W., PROC. SOC. EXP. BIOL. AND MED., 1937, v37, 107.

* The antisera were prepared by Miss E. Jane Tullius.