

feeding, standard anemic male rats gained  $5.5 \pm 0.14$  grams hemoglobin in six weeks, while females gained  $6.5 \pm 0.15$  grams of hemoglobin. However, when the rats had been made anemic in the presence of copper, presumably from the fourth or sixth week on, the hemoglobin gain for males and females over six weeks was only  $3.00 \pm 0.28$  and  $4.50 \pm 0.23$  grams, respectively. Our results (Table II) indicate that no signifi-

TABLE II  
SHOWING HEMOGLOBIN REGENERATION DURING CURATIVE PERIOD OF RATS MADE ANEMIC WITH AND WITHOUT COPPER

Amount of iron fed daily (mg)	Supplement to basal diet during depletion	No. of rats	Sex	Initial hemoglobin (gms/100 cc)	Gain in hemoglobin in 6 wks. curative period (gms/100 cc)
0.10	None	5	M	3.28	$4.72 \pm .22$
0.10	None	5	F	3.15	$5.76 \pm .19$
0.10	Copper	5	M	3.30	$4.60 \pm .24$
0.10	Copper	5	F	3.00	$5.83 \pm .20$

cant lessening of hemoglobin response occurred when copper had been fed throughout the entire depletion period as compared with hemoglobin response when regular depletion had been carried out. However, we noted a continuance of sex variation in response to iron feeding regardless of depletion technique. This does not corroborate the findings of Smith and Otis that sex variation diminished when copper was furnished during depletion and who believed that it would disappear entirely if a longer period of copper feeding were carried out.

It must be concluded from results obtained in this laboratory that supplementing the basal diet with copper in anemia production does not significantly affect the time nor severity of iron depletion, nor does it affect the hemoglobin response to subsequent iron feeding.

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## THE ABSORPTION OF SELENIUM BY CITRUS AND BY GRAPES

PLANTS differ greatly in their ability to accumulate selenium from the soil.<sup>1</sup>

This situation is of importance in connection with any public health hazards which may follow from the use of insecticidal sprays containing selenium. Hence the results of analyses of citrus fruit and grapes from plots which have been treated for several years with the commercial product Selocide and from nearby un-

treated plots may be of interest. This material corresponds in composition to the empirical formula  $(\text{KNH}_4\text{S})_5\text{Se}$  and is sold as a 30 per cent. aqueous solution.<sup>2</sup>

It was used for the control of the Pacific mite, *Tetranychus pacificus*, on grapes and of the citrus red spider, *Paratetranychus citri*, on citrus. The usual dilution was 1-800 on citrus and 1-600 on grapes. Samples of fruit were picked at random. All citrus fruit was washed in dilute nitric acid in order to remove adhering selenium from the exterior. Soil samples were taken at the edge of the trees or vines where the run-off of spray was heaviest. Only composite results for 0-36 inches depth are given in Table I:

TABLE I  
A. CITRUS

Plot	No. applications	Se in soil 0-36"	Se in fruit	
			skin	pulp
A (1)*	6 (1932-36)	ppm		ppm
AA (v.o.)†	None	0.91	0.09	0.07
B (v.o.)	6 (1933-36)	0.28	0.13	0.12
BB (v.o.)	none	0.46	0.47	0.07
C (1)	5 (1934-36)	0.29	0.07	0.03
CC (1)	none	0.80	0.31	0.06
D (v.o.)	3 (1935-36)	0.27	0.12	0.09
DD (v.o.)	none	0.61	0.22	0.12
E (v.o.)	3 (1933-36)	0.30	0.10	0.02
EE (v.o.)	none	0.27	0.17	0.07
F (1)	5 (1933-35)	0.35	0.09	0.04
FF (1)	none	0.93	0.18	0.02
G (1)	none	0.60	0.10	0.02
GG (1)	1 (1936)	0.21	0.22	0.03
H (v.o.)	none	0.12	0.08	0.01
HH (v.o.)	2 (1934)	0.53	0.09	0.03
	none	0.31	0.06	0.02

\* (1) = lemons.

† (v.o.) = Valencia oranges.

## B. THOMPSON SEEDLESS GRAPES

Field	No. applications	Se in soil 0-36"	Se in grapes	
			unwashed	washed
4	5 (1933-37)	0.49	1.80	0.64
4	2 (1933-34)	...	0.14	...
13-1	2 (1935-36)	...	0.23	...
4	none	0.25	0.11	...

On the basis of these results the following conclusions seem to be justified:

(1) Se occurs in all untreated soils tested at about 0.25 ppm.

(2) Se in soils of plots sprayed up to six times was always less than 1 ppm.

(3) The average Se content of the sprayed citrus fruits was: skin 0.21 ppm., pulp 0.06 ppm.

(4) The average Se content of the unsprayed citrus fruit was: skin 0.10 ppm., pulp 0.05 ppm.

(5) Grapes from vines sprayed during the current year contained over 0.6 ppm. Se, but the amount was much less when selenium was used in earlier years only.

(6) Neither citrus trees nor grape vines concentrate

<sup>1</sup> O. A. Beath *et al.*, *Jour. Am. Pharm. Assn.*, 26: 394-405, 1937; H. G. Byers, *U. S. Dept. Agr. Tech. Bul.* No. 482, 48 pp., 1935.

<sup>2</sup> C. B. Gnadinger, *Jour. Ind. Eng. Chem.*, 25: 633-7, 1933.

large amounts of selenium in their fruit as a result of spraying with selenium dissolved in potassium ammonium sulfide solution for the control of red spider.

A complete account of this and other work on

selenium carried out in the California Agricultural Experiment Station is in process of compilation.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### GROWTH SUBSTANCE DETERMINATIONS

THE Went *Avena*-coleoptile test and the *Cephalaria* test of Söding are the most sensitive ones that have been devised for quantitative hormone studies. These tests, however, are available only to a few laboratories which have facilities for the accurate regulation of temperature, humidity, etc.

The authors have developed a method of hormone determination which requires only the facilities available in every botanical laboratory. The manipulation is simple, no special apparatus is required, and the method is applicable over a wide range of growth substance concentrations. It may be used for detection of hormones at the low concentrations to which the *Avena* coleoptile responds, but so far as the method has been tested, does not appear to be available for the detection of minute differences within the low range of concentrations, as do the *Avena* and *Cephalaria* tests.

The new method depends on the fact that when etiolated seedlings of *Lupinus albus*, decapitated below the cotyledons, are exposed to light, growth ceases almost completely. However, when a growth substance is applied to the cut surface of the hypocotyl, elongation of the hypocotyl takes place in the presence of light, and this elongation is proportional to the concentration of the growth substance applied.

Some twelve or fourteen *Lupinus albus* seeds of approximately uniform size are planted in six-inch pots in sand. They are germinated in a dark room for six to seven days, or until the hypocotyls are seven to eight centimeters high. In the morning the pots are brought into the light, and the seedlings are selected for uniformity. The cotyledons are cut off with a razor blade, at the apex of the V-shaped notch which they make with the hypocotyl. A mark is made one centimeter below the cut, with India ink, and the growth substance is applied in an agar block, or in lanolin paste to the cut surface of the hypocotyl. The pots are placed under bell jars in order to insure high humidity and are exposed to full daylight. On the morning of the fourth day, the increase in length of the original centimeter segment is measured. Ten to fifteen test plants are used for each determination. With low concentrations of growth substances, control plants treated with lanolin or agar only are run in the same pot with the test plants. Indole-3-n-acetic acid in appropriate dilutions is used as a standard.

A straight-line relationship is obtained when high

concentrations of heteroauxin ( $100 \gamma - 0.01 \gamma$ ) are plotted logarithmically against increase in growth. However, in the lower range of heteroauxin concentration detected by the *Avena* test<sup>1</sup> ( $0.01 \gamma - 0.001 \gamma$ ) the curve rounds off.

The wide range of concentrations to which the hypocotyls respond should make this test especially applicable to the study of the growth-promoting activities of various substances that are not inactivated by light. Moreover, a test object whose physiological make-up is somewhat different from that of *Avena*<sup>2</sup> should prove of value in attempting to understand the mechanism of growth reactions.

The authors have had the benefit of Professor F. G. Gustafson's interest and advice in the studies, which will be described later in greater detail.

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### A BODY PLETHYSMOGRAPH FOR MEASURING RESPIRATORY VOLUMES WITH HIGH RESPIRATORY RATES

WHEN animals which are deficient in sweat glands, *i.e.*, dog, cat, rabbit, etc., are exposed to heat they develop a peculiar type of respiration called "heat polypnea" or "panting." The ventilation and respiratory rate increase, while the tidal volume decreases. With dogs respiratory rates may reach over 300 per minute. In measuring respiratory volumes of panting animals it is customary to use a tracheal canula or mask connected through valves to a recording system such as a respirometer or gas meter. Objections to this method include the following: (1) either anesthetics must be used or the respiratory pattern becomes subject to artificial alterations due to pressure stimuli, pain, etc.; (2) the dead space of the apparatus does not match that of the normal animal; (3) canulae impede air movement; (4) the purpose of panting is to blow air over the moist surface of mouth, tongue and pharynx for purposes of evaporation and this cooling mechanism is lost to the cannulated animal; (5) moving mechanical systems have the disadvantages of having appreciable inertia and of giving false records when the respiratory period approaches or becomes less than the natural period of the moving system. In order to

<sup>1</sup> George S. Avery, Jr., Paul R. Burkholder and Harriet B. Creighton, *Am. Jour. Bot.*, 24: 226-232, 1937.

<sup>2</sup> Sam Granick and H. W. Dunham, *Papers, Mich. Acad.*, 22: 69-78, 1936.