THE DEVELOPMENT OF STEROLS IN THE BEAN PLANT, (PHASEOLUS VULGARIS)

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Lou Cornelia Key

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THE DEVELOPMENT OF STEROLS IN THE BEAN PLANT, (PHASEOLUS VULGARIS)

By

Lou Cornelia Key

An Abstract

Submitted to the College of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

A study was made of the formation of free and total sterols during different stages of growth of the bean and bean plant, Phaseolus vulgaris, variety Michilite. The effects of different sample preparations, namely fresh tissue, air-dry tissue and oven dry tissue, were studied. During the last harvest, the roots were gathered and analyzed for free and total sterols. Moisture determinations were made on the fresh tissue and on the air-dry tissue and all the results were reported on a moisture free basis.

The "crude lipid" material was extracted with Skelly-solve B, and an estimation of lipid content was made. Aliquots of the lipid material were passed through an adsorption column, containing Hyflo Super-Cel and activated Magnesia, for free sterol analysis and aliquots were saponified for total sterol analysis. Digitonides of the sterols were precipitated from the eluates and from the unsaponifiable extracts. The digitonides were washed with 30% ethanol and with ethyl ether and then oxidized with sulfuric acid-potassium dichromate reagent. The excess dichromate was titrated by means of 0.1N ferrous ammonium sulfate solution. The mgm. of sterol present was read from working curves prepared by plotting known amounts

of sterols against the ml. of ferrous solution equivalent to the dichromate used for oxidation.

The results of the study showed that the bean and bean plant contained little or no combined sterols; however, the bean roots or the nodules or both contained combined sterols as well as free sterols.

The sterol content of the bean plants increased rapidly up to about the 55th day of growth, then decreased until between the 60th and 65th day of growth, and later increased to about the level obtained about the 55th day. During this decrease in the sterol content of the plant, there was a rapid increase in the sterol content of the bean. It would appear that the bean plant, with beans can synthesize sterols at any stage of development since the increase in the sterol content of the beans more than accounts for the decrease in the sterol content of the plant alone. The sterol content of the beans increased continuously but the most rapid increase was during the first two weeks of growth of the bean.

The sterol development of the beans paralleled that of the lipid development in that the most rapid formation was during the first two weeks of growth, and both showed a slow but continuous development to reach a maximum in the mature bean. The lipid content of the plant tissue increased up to between the 50th and 55th day of growth, after which there was a decrease and later a slight increase.

The results for the sterol analysis of the fresh bean plant tissue and that which was previously air dried during all stages of development were similar, but some destruction of sterols in oven dry tissue was apparent. However, the results for the sterol analysis of fresh bean tissue and that which was oven dried were similar, indicating the beans may contain different sterols from those in the plant.

Attempts to air-dry the beans were unsuccessful.

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CONTENTS

I. INTRODUCTION

It has been known for a long time that the vegetable sterols can be synthesized by both the lower and the higher plants, however, little attention has been given to the sterols of the vegetative portion of green plants. With an increase in the use of certain sterols for the production of pharmaceuticals, a study of the development of these sterols in plants seemed profitable. The Michillite bean plant was selected for this study and the development of free sterols as well as total sterols was followed. An analysis for the presence of combined and free sterols in the roots of the bean plants was also carried out. There have been no comparisons made on the preliminary treatment of samples prior to analysis, so it was thought that a comparison using air dry, oven dry and fresh tissue would be interesting.

II. HISTORICAL

Waghorne and Ball (1) traced the development of free sterols in corn leaves using air dry tissue. They observed that the sterols are retained in the leaf for a much longer period than the lipids, but finally they too begin to disappear. They also observed that the corn leaf has the ability to synthesize sterols up to about the 75th day of growth but that following this period the synthesis is definitely limited and does not parallel their destruction. Their study indicated that the rate of synthesis of the sterols may not quite keep pace with the rate of growth of the corn leaf since there appeared to be a slight decrease in the percentage value.

Blair, Mitchell, and Silker (2) made a study of the factors which influence the sterol content of alfalfa, bromegrass, and wheat plants using oven dry tissue. They observed that the free sterols decreased as the plants approached maturity, while, the combined sterols were detected in alfalfa at all times during growth up to the early bloom stage. They found that the wheat plant contained no combined sterols until heads began to appear, at which time those accounted for 25% of the sterols. Their study indicated that fertilization of bromegrass with NH_4NO_3 caused an increase in both free and combined sterols.

III. EXPERIMENTAL

Procedure

Preliminary treatment of samples prior to analysis

Samples were taken at two o'clock on afternoons of clear days whenever possible. The portion of the bean plants above ground was immediately taken to the laboratory where dead plant leaves and extraneous matter were removed. The plants were then counted, weighed and divided into three parts for the three different preliminary treatments, namely fresh tissue, air-dry tissue, and oven dry tissue. When beans appeared on the plants they were picked off and treated just as the plants, except that attempts to air dry the beans were unsuccessful. Even when they were spread on a table and the air blown over a hot plate spoilage occured before the beans were dry enough to grind.

The part for the fresh tissue treatment was cut into small pieces with scissors and mixed. Twenty-five gm. duplicate samples were then transferred to 600 ml. beakers and enough 95% ethanol was added to bring the alcoholic content to about 80%. The tissue was then boiled for 15 minutes on a steam bath. The fresh tissue was then transferred to 33 x 94 mm. paper thimbles by fitting the extraction thimbles

into glass filter funnels held in turn in clean suction flasks. The tissue was washed with a jet of hot 95% ethanol and then covered with a piece of filter paper.

The portion for air dry treatment was spread on a table and allowed to dry at room temperature until brittle. This usually took from two to three days.

The portion for oven dry treatment was allowed to dry for 18 hours in an electric oven at 95-100°C.

After drying the samples were ground in a semimicro Wiley mill to pass through a 40-mesh sieve and then mixed thoroughly.

A portion of the oven dry tissue was transferred to a weighing bottle and dried again for about 16 hours in an electric oven at 95-100°C. The material was then allowed to cool in a desiccator, after which ten gram samples were weighed and transferred to 33 x 94 mm. paper thimbles and then covered with a piece of filter paper.

During the last harvest of bean plants the roots were gathered after removal of the tops. The roots were very hard to handle and contained a mass of very fine network and the ones in the same pot were practically impossible to separate without tearing. They were hard to wash and grind. The roots contained a considerable number of nodules, some of which were lost while washing. The roots were washed with

distilled water, spread on a table to dry overnight, after which they were counted as well as possible and weighed. The roots were then treated in the same manner as the oven dry plant tissue already described.

Samples of various portions of the plant were weighed out for moisture determinations at the same time as those for analysis. Moisture content of the fresh plant material and of the air-dry plant material was measured by placing duplicate five gram samples of each in special aluminum pans and drying at 100°C until constant weight in a Brabender Moisture Tester. Percent moisture was obtained by multiplying the Brabender scale reading by two since the moisture tester was calibrated for ten gram samples.

Extraction of Sterols. The paper thimbles were placed in the extraction section of continuous Soxhlet extractors each of which was previously equipped with a three cm. length of large-bore glass tubing. Three different 18 hour extraction periods were made with Skellysolve B. The fresh material was freed of Skellysolve B and finely ground before subjecting to the third and last extraction period.

One ml. of 0.02% Hydoquinone (dissolved in 95% ethanol) was added to the combined extracts which were evaporated to dryness on a steam bath in an open beaker in a swiftly moving current of air. When the solvent apparently was evaporated the beakers were put in a vacuum oven at 30°C and dried for 16

hours. The dried "crude lipid" material was then treated with Skellysolve B and filtered into weighed 50 ml. beakers. The solvent was evaporated as before and the beakers were put in a vacuum oven at 80°C and allowed to come to constant weight for an estimation of the lipid content.

The dried extracts were transferred with Skellysolve B to 25 ml. volumetric flasks and made to volume and mixed. Partial purification for precipitation was done by use of Wall and Kelly's procedure (3).

Liberation of combined sterols. In order to liberate the combined sterols, 10 ml. aliquots of the Skellysolve extract were refluxed for 30 minutes with 5 ml. of 10% KOH in 95% ethanol. The saponification mixture was transferred to a small separatory funnel by using Skellysolve B and 95% ethanol alternately. The alcohol layer was removed and extracted three times with Skellysolve B and the washings were combined with the original Skellysolve solution. The combined solution was then extracted four or five times with 90% methanol to remove carotenols, traces of chlorophyll and alkali, after which it was transferred to 250 ml. beakers.

Adsorption of extract for free sterol analysis. In order to determine the free sterols 10 ml. aliquots of the Skellysolve extract were taken at the same time as those to be saponified and run through an adsorption column (15 x 2 cm.),

filled with a mixture of three parts of Hyflo Super-Cel (Johns-Manville Corp.) and one part activated Magnesia No. 2641 (Westvaco Chloride Products Co.). The sterols as well as carotenes were eluted with 50 ml. of 5% acetone in Skellysolve B followed by 50 ml. of 10% acetone in Skellysolve B. The eluate was caught in 250 ml. beakers, placed in a vacuum desiccator and reduced pressure obtained by a water pump.

Precipitation and oxidation of digitonides. Following the adsorption of the samples for free sterol analysis and the saponification for total sterol analysis, the precipitation and subsequent oxidation was done by use of Waghorne and Ball's procedure (4). The eluates as well as the unsaponifiable extracts were evaporated just to dryness on a well ventilated steam bath. The residues were cooled, taken up with Skellysolve B and filtered into 25 ml. volumetric flasks and were made up to volume and mixed. Five ml. aliquots were transferred to 15 ml. centrifuge tubes and evaporated to dryness over vapors of rapidly boiling 95% ethanol as illustrated by Waghorne and Ball. The sides of the tubes were washed thoroughly by the use of 5 ml. of absolute ethanol, after which the centrifuge tubes were replaced in the boiling ethanol vapors. After the residue had dissolved, 2 ml. of 1%

digitonin in 80% ethanol and 1.25 ml. of distilled water were added. The contents were mixed thoroughly by means of a glass thread which in turn was rinsed with 80% ethanol. The tubes were heated for a minute longer and then placed in pint preserving jars which contained about one quarter inch of 80% ethanol in the bottom (5). The jars were sealed by means of a screw top and precipitation was allowed to occur overnight.

After overnight precipitation, any precipitate sticking to the walls of the tube was loosened by rubbing with the glass thread and rinsing down with 80% ethanol. The tubes were then centrifuged at about 3000 r.p.m. for one-half hour and the supernatant liquid was removed by means of a capillary tube and gentle suction. The precipitate was washed once with 3 ml. of 80% ethanol, and thoroughly mixed by the aid of the fine glass rod and the glass rod rinsed. The tubes were centrifuged and the supernatant liquid removed as before. The precipitate was then washed at least twice with ethyl ether using the above technique.

After the final removal of the ether, the tubes were allowed to stand overnight in a covered beaker at room temperature. Drying was then completed by placing them in an electric oven at 90° to 95°C for about 1 hour. The tubes were then cooled and exactly 4 ml. of sulfuric acid-potassium dichromate reagent was added. The tubes were then suspended

in a steam bath to a depth approximately 0.25 inch above the surface of the reagent. This was accomplished by slipping large corks over the tubes to support them on the steam bath cover. The tubes were left stationary for 3.5 hours, after which they were inspected and any precipitate clinging to the sides of the tube was rinsed down by gentle agitation. Heating was then continued for another 0.5 hour.

The contents of the tubes were then quantitatively rinsed into beakers with 40 to 50 ml. of distilled water. One drop of o-phenanthroline ferrous complex indicator (6) was added, and the excess dichromate was titrated by means of 0.1N ferrous ammonium sulfate solution, previously run through a lead reductor just before titration (7).

Preparation of working curve. Working curves for this analysis were prepared by plotting the mgm. of sterol present against the ml. of ferrous solution equivalent to the dichromate used for the oxidation (Figures 1 and 2). The titration values on which Figures 1 and 2 are based are to be found in Tables IA and IB. The stigmasterol used in this work was prepared from crude soybean sterols by recrystallizing both as the acetate and free sterol a total of fourteen times; M.P. 162-163°C. The sitosterol used for this work came from Nutritional Biochemicals Corporation and was recrystallized from absolute ethanol four times; M.P. 129-131°C.

TABLE IA

OXIDATION OF KNOWN AMOUNT OF STIGMASTEROL

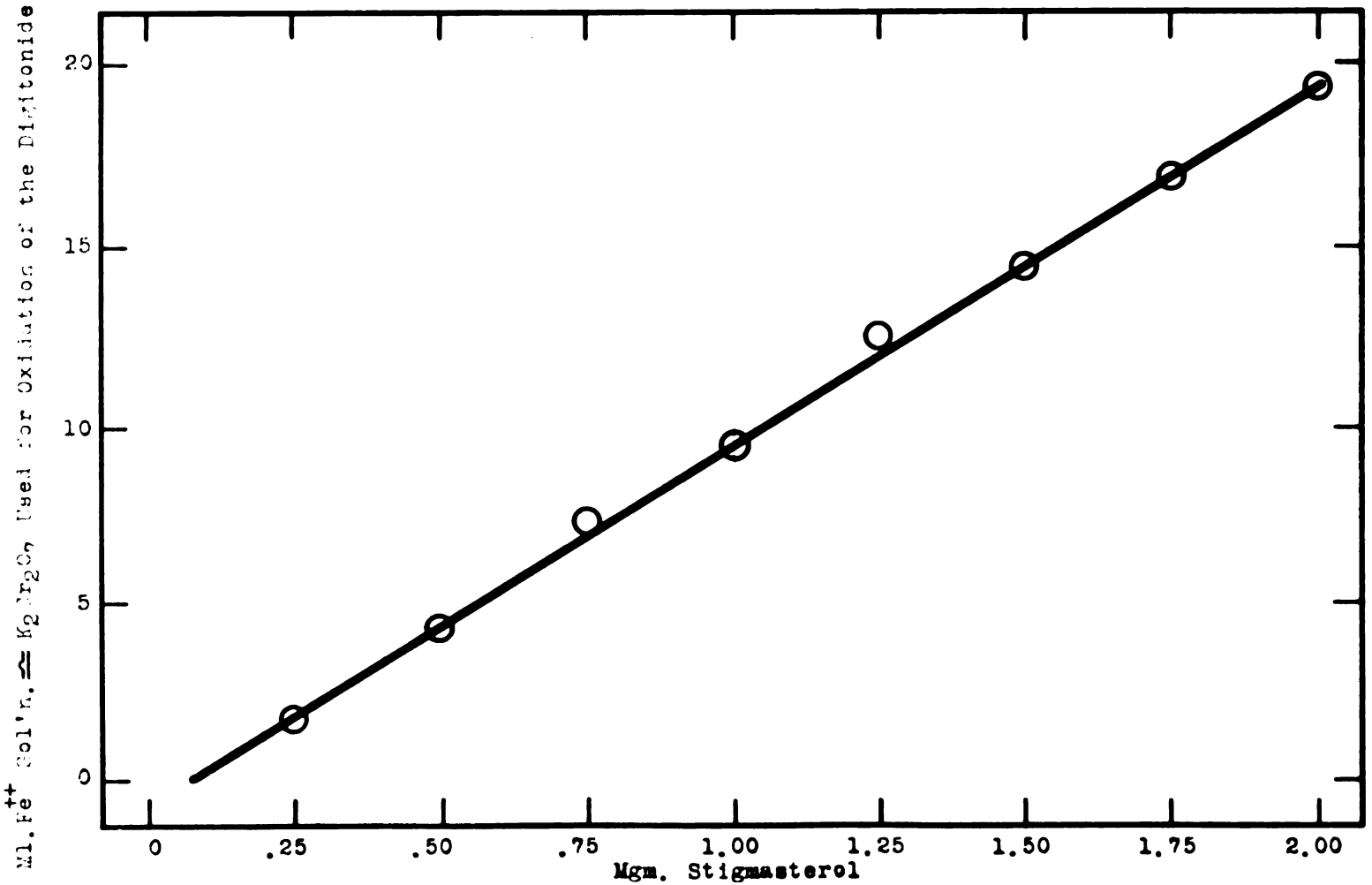
Stigmasterol Present M.m.	Ml.Fe^{++} Sol'n. Excess $Cr_2O_7^{=}$	Average Ml. Fe^{++} Sol'n. Excess $Cr_2O_7^{=}$	Ml.Fe^{++} Sol'n. $Cr_2O_7^{=}$ Used for Oxidation
0.00	23.50 23.57 23.60 23.60	23.57	---
0.25	21.90 21.85 21.84	21.86	1.71
0.50	19.55 19.26	19.41	4.16
0.75	16.55 16.33 16.30	16.39	7.18
1.00	14.33 14.45	14.39	9.18
1.25	10.96 11.07 11.21 11.55	11.20	12.37
1.50	9.47 9.30	9.39	14.18
1.75	6.85 6.81	6.83	16.74
2.00	4.61 4.00	4.31	19.26

TABLE IB

OXIDATION OF KNOWN AMOUNT OF SITOSTEROL

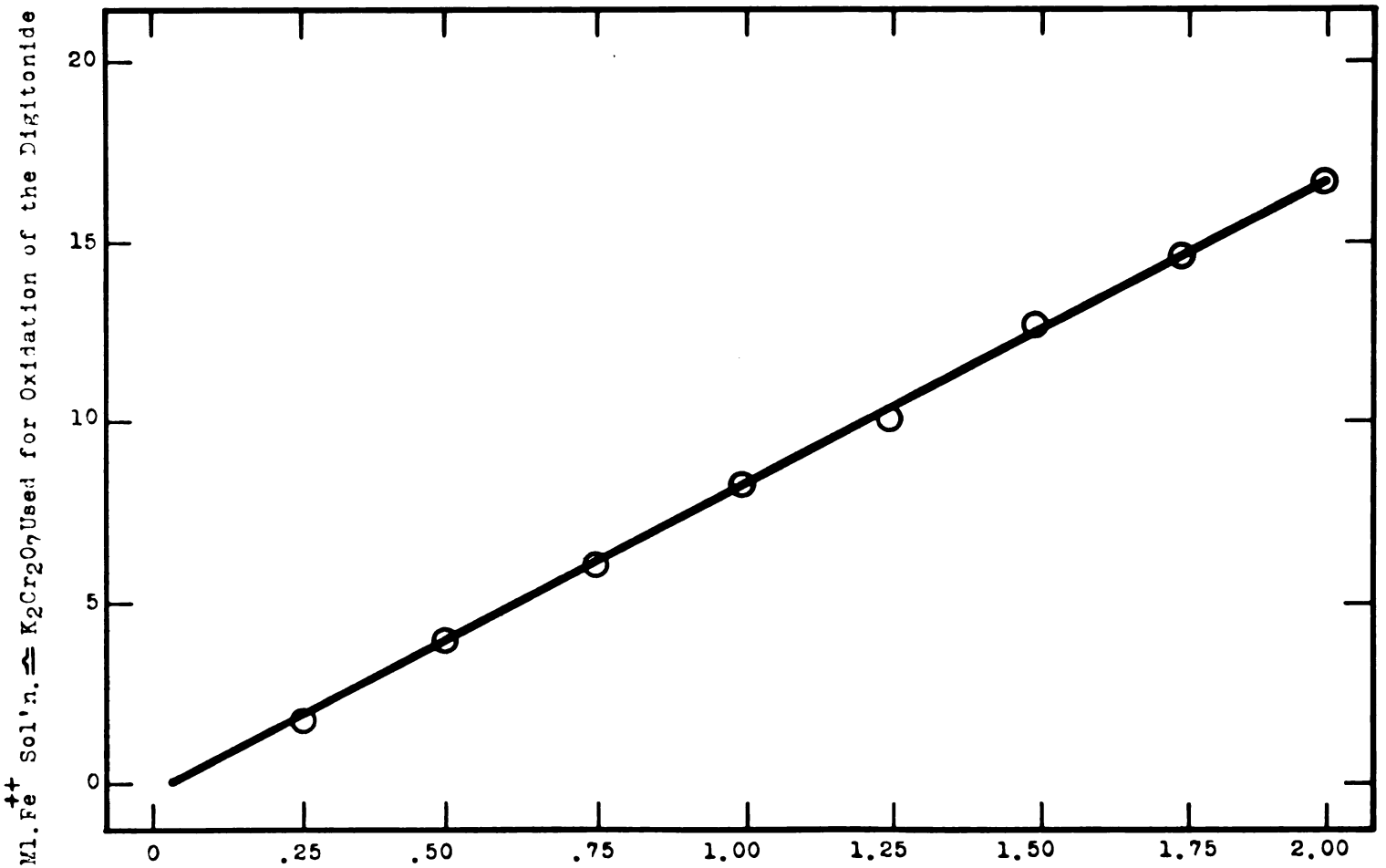
Sitosterol Present Mgm.	Ml. Fe^{++} Sol'n. Excess $Cr_2O_7^=$	Average Ml. Fe^{++} Sol'n. Excess $Cr_2O_7^=$	Ml. Fe^{++}Sol'n. Cr_2O_7 Used for Oxidation
0.00	23.04 23.26	23.15	---
0.25	21.50 21.50	21.50	1.65
0.50	19.21 19.19	19.20	3.95
0.75	17.16 17.16	17.16	5.99
1.00	15.08	15.08	8.07
1.25	13.00 13.27	13.14	10.01
1.50	10.72 10.52	10.62	12.53
1.75	8.78 8.78	8.78	14.37
2.00	6.84 6.55	6.70	16.45

FIG. 1



WORKING CURVE FOR STIGMASTEROL

FIG. 2



WORKING CURVE FOR SITOSTEROL

IV. RESULTS AND DISCUSSION

The weight growth curve (Figure 3), plotted from data in Table II, shows that the plant reaches maximum growth at about the same time the growth of the beans starts reaching a maximum. The beans reach maximum growth much more quickly than the plants, and both fall off rather slowly to reach a minimum at about the same time.

It can be noted from Tables III and IV and Figures 4 and 5 that the lipid content of the plant increases fairly rapidly until between about 50 and 55 days of age, after which it decreases, during which time there is a rapid build up of lipid in the fast growing young beans. The lipid content of the air-dry plant tissue and the oven dry plant tissue then starts building up again, during which time there is not as rapid development of lipid in the beans as during their first two weeks of growth, to reach a maximum in the mature plant. The lipid content of the fresh plant tissue builds up again very slowly and does not reach the peak which was obtained between 45 and 55 days of age. It can be observed that the lipid content of the bean continues to rise slowly, even after its growth period has culminated, to reach a maximum in the mature bean. The lipid content of the oven dry plant tissue

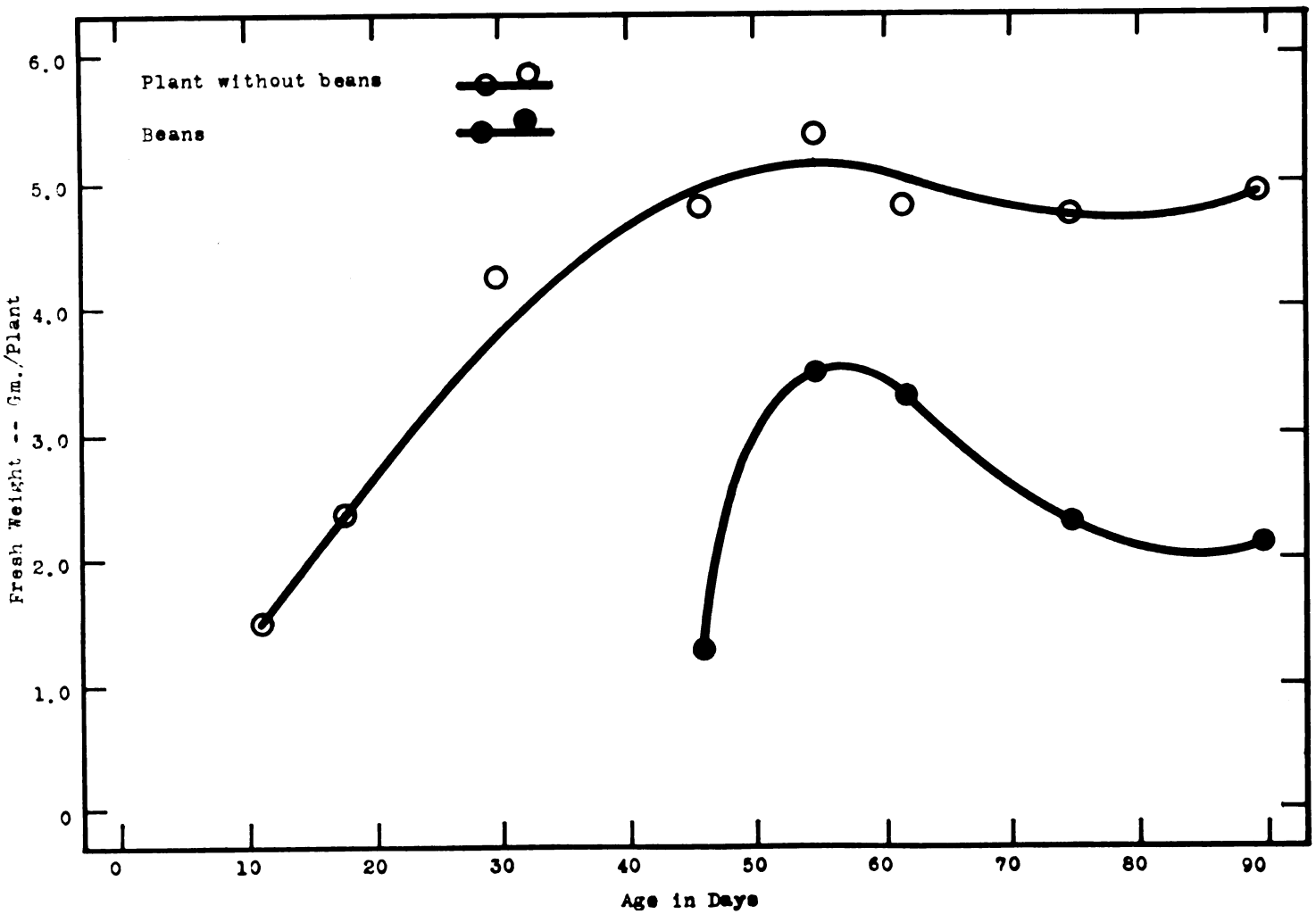
TABLE II

GROWTH DATA OF PLANT AND BEANS

Age of Plant Days	No. of Plants from which Beans Were Picked	Total No. of Beans	Total Weight of Beans Gms.	(1) Fresh Weight per Bean Gms.	Fresh Weight of Beans per Plant Gms.	(2) Fresh Weight per Plant without Beans Gms.
11						1.52
18						2.40
30						4.26
46	243	342	300.17	0.88	1.24	4.81
55	219	330	764.79	2.03	3.49	5.38
62	216	335	709.80	2.12	3.29	4.80
75	202	309	461.49	1.49	2.28	4.74
90	202	373	421.55	1.11	2.09	4.89

(1) Averages of at least 3 different determinations
(2) Averages of at least 6 different determinations

FIG. 3



GROWTH OF PLANT WITHOUT BEANS AS COMPARED TO THE GROWTH OF THE BEANS

TABLE III

COMPARISON OF LIPID CONTENT OF PLANT WITH GROWTH AND WITH PRELIMINARY TREATMENTS

Age of Plant Days	Fresh Weight per Plant Gms.	(1) % Moisture	Dry Weight per Plant Gms.	(1,2) % Lipid Fresh Tissue	(1,2) % Lipid Air-dry Tissue	(1,2) % Lipid Oven Dry Tissue	(1,2) Mgm. Lipid per Plant Fresh Tissue	(1,2) Mgm. Lipid per Plant Air-Dry Tissue	(1,2) Mgm. Lipid per Plant Oven Dry Tissue
11	1.52	89.80	.155	12.94	2.91	2.73	20.06	4.51	4.23
18	2.40	88.40	.278	10.08	3.37	2.87	28.03	9.37	7.98
30	4.26	87.05	.552	9.15	3.43	2.21	50.51	18.93	12.20
46	4.81	79.32	.995	6.74	2.33	2.06	67.06	23.18	20.50
55	5.38	83.32	.897	7.40	3.25	2.51	66.38	29.15	22.51
62	4.80	83.82	.777	7.57	2.92	2.03	58.82	22.69	15.77
75	4.74	77.20	1.08	5.35	3.29	2.12	57.78	35.53	22.90
90	4.89	77.50	1.10	5.69	3.46	2.39	62.59	33.06	26.29

(1) Averages of at least duplicate determinations.
(2) Reported on moisture free basis.

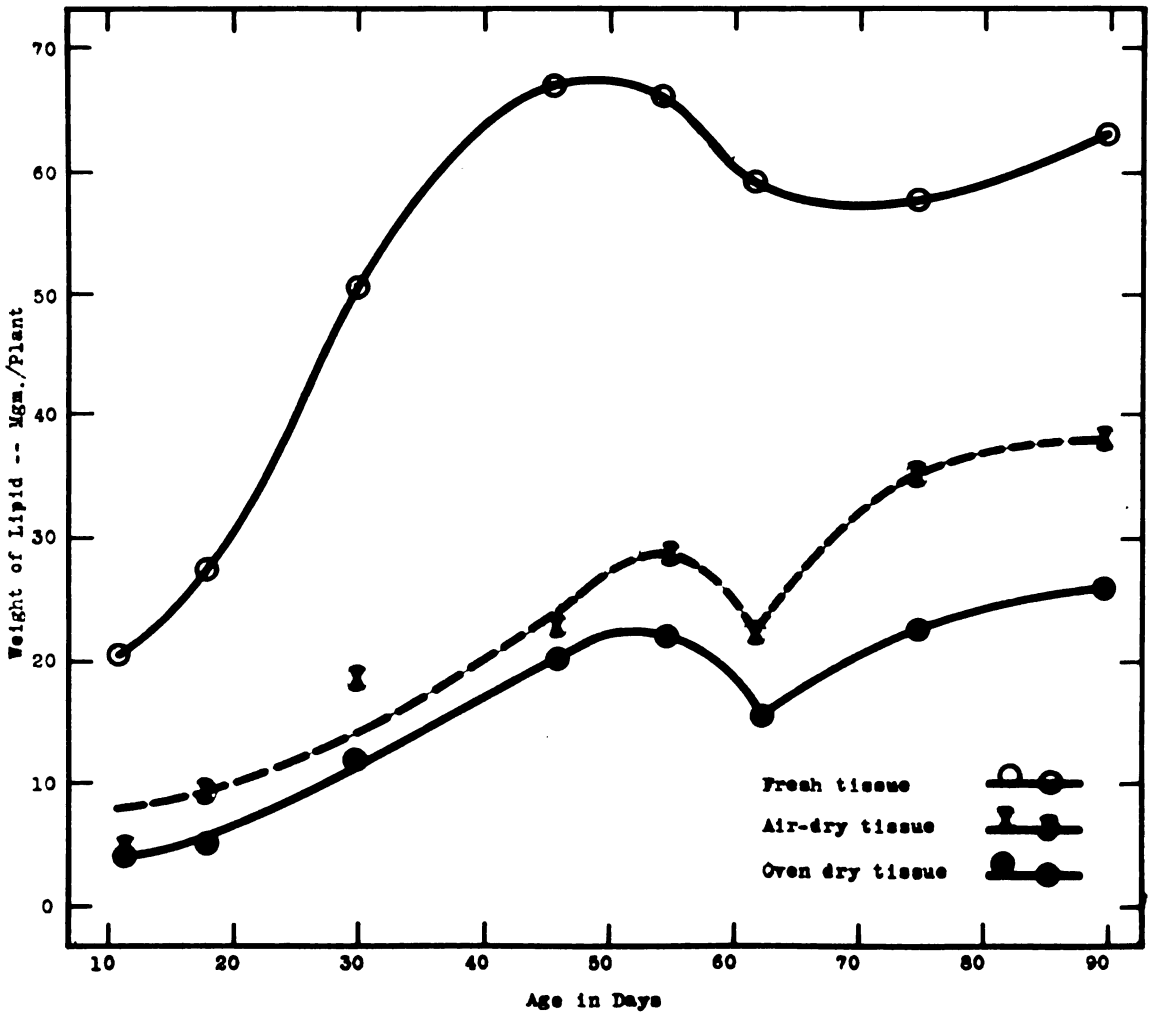
TABLE IV

COMPARISON OF LIPID CONTENT OF BEANS WITH GROWTH AND WITH PRELIMINARY TREATMENTS

Age of Plant Days	Fresh Weight of Beans per Plant Gms.	(1) % Moisture	Dry Weight of Beans per Plant Gms.	(1,2) % Lipid Fresh Tissue	(1,2) % Lipid Oven Dry Tissue	(1,2) Mgm. Lipid per Bean Fresh Tissue	(1,2) Mgm. Lipid per Bean Oven Dry Tissue	(1,2) Mgm. Lipid in Beans per Plant Fresh Tissue	(1,2) Mgm. Lipid in Beans per Plant Oven Dry Tissue
46	1.24	89.85	.126	3.31	1.58	2.95	1.41	4.17	2.00
55	3.49	83.12	.589	2.11	1.43	7.22	4.90	12.40	8.41
62	3.29	76.19	.783	2.76	1.79	13.94	9.04	21.61	14.02
75	2.28	44.90	1.256	1.69	1.44	13.84	11.82	21.17	18.08
90	2.09	24.17	1.585	1.64	1.35	13.77	11.37	25.92	21.40

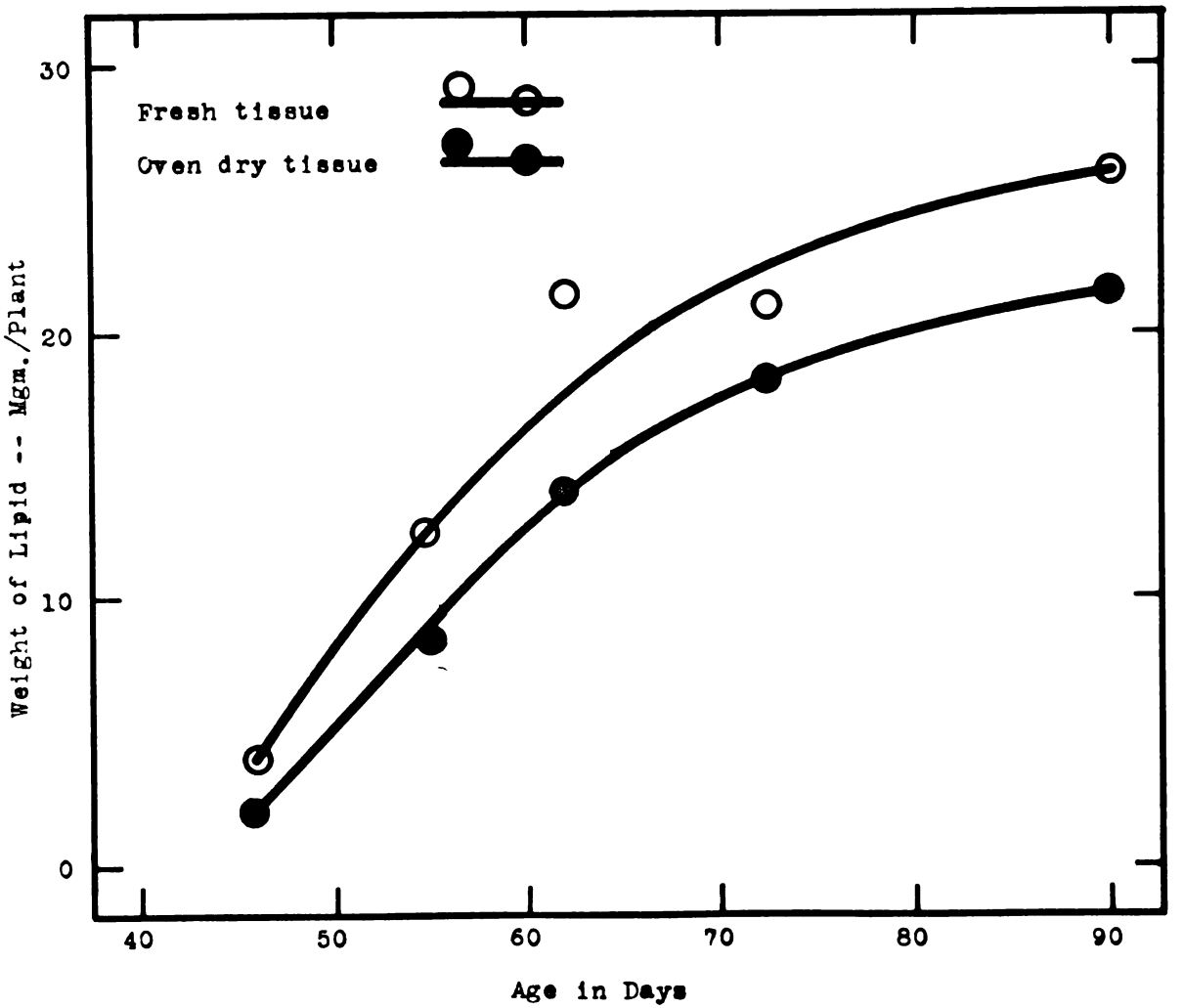
(1) Averages of at least duplicate determinations.
(2) Reported on moisture free basis.

FIG. 4



LIPID CONTENT OF PLANT WITHOUT BEANS AS INFLUENCED BY SAMPLE TREATMENT

FIG. 5



LIPID CONTENT OF BEANS AS INFLUENCED BY TREATMENT

and air-dry plant tissue is considerably lower than that for the fresh tissue. This difference can be attributed to the chlorophyll, carotenes, etc. destroyed by the preliminary air-dry and oven dry treatments.

A number of interesting observations can be made from Tables V, VI and Figures 6, 7, 8, 9. The sterol content of the plant in all of the three different preliminary treatments, including the free and total sterol analysis, increases rapidly up to about the 55th day of growth, after which it decreases rapidly until between the 60th and 65th day of growth, after which it increases again. Whereas, the sterol content of the beans increases continuously, the most rapid increase appears to be during the first two weeks of growth of the very young bean. The sterol content of the plant increases during about the same period as the lipid content increases and also decreases at about the same period. It is interesting to note (Figures 5, 8, 9) that the development of sterols in the bean parallels the lipid development and that the most rapid formation is during about the first two weeks of growth of the very young bean. In both cases there is continuous development to reach a maximum in the mature bean.

There appears to be little significant difference between the free sterol content and the total sterol content of the beans or plants during any stage of development, so this would

indicate that the bean and bean plant contain little or no combined sterols. However it can be seen from Table VII that the total sterol content of the roots is twice that for the free sterol content, hence, this would indicate that half of the sterol content is in the combined form. It is not known whether the sterol content of the roots is actually contained by the roots or by the nodules on the roots or by both.

In the case of the bean plants the analysis (Figures 6, 7) for both the free and total sterols indicate that the values for the oven dry preliminary treatment are significantly lower than for the fresh and air dry tissue. Whereas, in the case of the beans there is no significant difference between the values for the oven dry and fresh tissue, Figures 8, 9. Perhaps the beans contain different sterols from those in the plant, and some of those in the plant are destroyed by the heat in the oven dry treatment. If this is the case then there would have to be synthesis of sterols going on in the bean as well as in the plant. The fact that there is an increase in the development of sterols in the plant during a period when there is an increase in the development of sterols in the bean would also indicate synthesis of sterols in both the plant and bean. There appears to be no significant difference between the fresh and air dry preliminary treatments as can be seen from Table V and Figures 6, 7. As can be seen from

Tables V and VI the bean plant containing beans apparently can synthesize sterols at any stage of development since the increase in the sterol content of the bean can more than account for the decrease in the sterol content of the plant.

TABLE V

COMPARISON OF FREE AND TOTAL STEROL CONTENTION OF PLANTS
AGED WITH FRESH AND WITH PRELIMINARY TREATMENTS

Age of Plant Days	Preliminary Treatment	Mgm. Free Sterol per Gm. Tissue	Mgm. Total Sterol per Gm. Tissue	(1) % Moisture	Mgm. Free Sterol per Gm. Tissue H_2O Free Basis	Mgm. Total Sterol per Gm. Tissue H_2O Free Basis	Dry Weight per Plant	(2) Mgm. Free Sterol per Plant	(2) Mgm. Total Sterol per Plant
11	Fresh Tissue	.190	.269 .267	89.30	1.765	2.637 2.613	.155	.274	.409 .406
11	Air Dry Tissue	1.720 1.651	1.802 1.798	5.80	1.826 1.735	1.913 1.909	.155	.283 .277	.297 .296
11	Oven Dry Tissue	.916 .910	1.094 1.114	-	.916 .910	1.094 1.114	.155	.147 .146	.170 .173
13	Fresh Tissue	.149 .143	.191 .191	88.40	1.207 1.233	1.647 1.647	.273	.336 .343	.453 .456
13	Air Dry Tissue	1.023 1.067	1.134 1.103	6.00	1.094 1.114	1.206 1.173	.273	.304 .310	.335 .326
13	Oven Dry Tissue	.670 .661	.844 .845	-	.670 .661	.844 .845	.273	.136 .124	.235 .235
30	Fresh Tissue	.163 .151 .153 .161	.157 .157	87.05	1.259 1.166 1.220 1.243	1.212 1.212	.552	.695 .644 .673 .696	.669 .669
30	Air Dry Tissue	1.021 1.035	.890 .848	8.24	1.113 1.123	.970 .924	.552	.614 .623	.535 .510

TABLE V

COMPARISON OF FREE AND TOTAL STEROL COMPOSITION OF PLANTS ALONE WITH GROWTH AND WITH PRELIMINARY TREATMENTS

Age of Plant Days	Preliminary Treatment	Mgm. Free Sterol per Gm. Tissue	Mgm. Total Sterol per Gm. Tissue	(1) % Moisture	Mgm. Free Sterol per Gm. Tissue H_2O Free Basis	Mgm. Total Sterol per Gm. Tissue H_2O Free Basis	Dry Weight per Plant	(2) Mgm. Free Sterol per Plant	(2) Mgm. Total Sterol per Plant
11	Fresh Tissue	.180	.269 .267	89.80	1.765	2.637 2.618	.155	.274	.409 .406
11	Air Dry Tissue	1.720 1.681	1.802 1.798	5.80	1.826 1.785	1.913 1.909	.155	.283 .277	.297 .296
11	Oven Dry Tissue	.946 .940	1.094 1.114	-	.946 .940	1.094 1.114	.155	.147 .146	.170 .173
18	Fresh Tissue	.140 .143	.191 .191	88.40	1.207 1.233	1.647 1.647	.278	.336 .343	.458 .458
18	Air Dry Tissue	1.028 1.047	1.134 1.103	6.00	1.094 1.114	1.206 1.173	.278	.304 .310	.335 .326
18	Oven Dry Tissue	.670 .661	.844 .845	-	.670 .661	.844 .845	.278	.186 .184	.235 .235
30	Fresh Tissue	.163 .151 .158 .161	.157 .157	87.05	1.259 1.166 1.220 1.243	1.212 1.212	.552	.695 .644 .673 .686	.669 .669
30	Air Dry Tissue	1.021 1.035 .205	.890 .848	8.24	1.113 1.128 .991	.970 .924	.552	.614 .623 .986	.535 .510
46	Air Dry Tissue	.943 .959	.977 1.040 1.055 1.101	7.39	1.018 1.036	1.055 1.123 1.139 1.189	.995	1.01 1.03	1.05 1.12 1.13 1.18
46	Oven Dry Tissue	.559 .595	.544 .534	-	.559 .595	.544 .534	.995	.556 .592	.541 .531
55	Fresh Tissue	.196 .197	.232 .237	83.32	1.175 1.181	1.391 1.421	.897	1.05 1.06	1.25 1.27
55	Air Dry Tissue	1.212 1.185	1.201 1.209	8.29	1.322 1.292	1.310 1.318	.897	1.19 1.16	1.18 1.18
55	Oven Dry Tissue	.799 .794	.946 .920	-	.799 .794	.946 .920	.897	.717 .712	.849 .825
62	Fresh Tissue	.196 .203	.232 .224	83.82	1.211 1.255	1.434 1.384	.777	.941 .975	1.11 1.08
62	Air Dry Tissue	.965 .982	1.175 1.153	7.13	1.039 1.057	1.265 1.242	.777	.807 .821	.983 .965
62	Oven Dry Tissue	.729 .727	.661 .704	-	.729 .727	.661 .704	.777	.566 .565	.514 .547
75	Fresh Tissue	.207 .205	.232 .234	77.20	.908 .899	1.018 1.026	1.08	.981 .971	1.10 1.11
75	Air Dry Tissue	.940 .909	.966 .939	9.49	1.039 1.004	1.067 1.037	1.08	1.12 1.08	1.15 1.12
75	Oven Dry Tissue	.663 .648	.758 .750	-	.663 .648	.758 .750	1.08	.716 .700	.819 .810
90	Fresh Tissue	*.159 .164	.243 .239	77.50	.707 .729	1.080 1.062	1.10	*.778 .802	1.19 1.17
90	Air Dry Tissue	.915 .915	1.003 1.031	10.78	1.026 1.026	1.124 1.156	1.10	1.13 1.13	1.24 1.27
90	Oven Dry Tissue	.789 .755	.858 .856	-	.789 .755	.858 .856	1.10	.868 .831	.944 .942

(1) Average of at least duplicate determinations
(2) Reported on moisture free basis
* Very light colored eluate, possibly some remained on column

TABLE VI

COMPARISON OF FREE AND TOTAL STEROL COMPOSITION OF BEANS WITH GROWTH AND WITH PRELIMINARY TREATMENTS

Age of Plant Days	Preliminary Treatment	Dry Weight of Beans per Plant Gms.	Mgm. Free Sterols per Gm. Tissue	Mgm. Total Sterol per Gm. Tissue	(1) % Moisture	Mgm Free Sterol per Gm. Tissue H_2O Free Basis	Mgm. Total Sterol per Gm. Tissue H_2O Free Basis	(2) Mgm. Free Sterol in Beans per Plant	(2) Mgm. Total Sterol in Beans per Plant
46	Fresh Tissue	.126	.051 .057	.051 .050	89.85	.502 .562	.502 .493	.063 .071	.063 .062
46	Oven Dry Tissue	.126	.617 .555 .568 .601	.567 .558	-	.617 .555 .568 .601	.567 .558	.078 .070 .072 .076	.071 .070
55	Fresh Tissue	.589	.104 .103	.096 .101	83.12	.616 .610	.569 .598	.363 .359	.335 .352
55	Oven Dry Tissue	.589	.589 .595	.526 .558	-	.589 .595	.526 .558	.347 .350	.310 .329
62	Fresh Tissue	.783	.153 .160 .157 .155	.114 .114	76.19	.643 .672 .659 .651	.479 .479	.503 .526 .516 .510	.375 .375
62	Oven Dry Tissue	.783	.526 .513	.473 .503	-	.526 .513	.473 .503	.412 .402	.370 .394
75	Fresh Tissue	1.256	.292 .304 .289 .300	.258 .263 .253 .254	44.90	.530 .552 .525 .544	.468 .477 .459 .461	.666 .693 .659 .683	.588 .599 .577 .579
75	Oven Dry Tissue	1.256	.468 .468	.446 .458	-	.468 .468	.446 .458	.588 .588	.560 .575
90	Fresh Tissue	1.585	.441 .425 .420 .452	.323 .332	24.17	.582 .560 .554 .596	.426 .438	.922 .888 .878 .945	.675 .694
90	Oven Dry Tissue	1.585	.446 .446	.441 .440	-	.446 .446	.441 .440	.707 .707	.699 .697

(1) Average of at least duplicate determinations
(2) Reported on moisture free basis

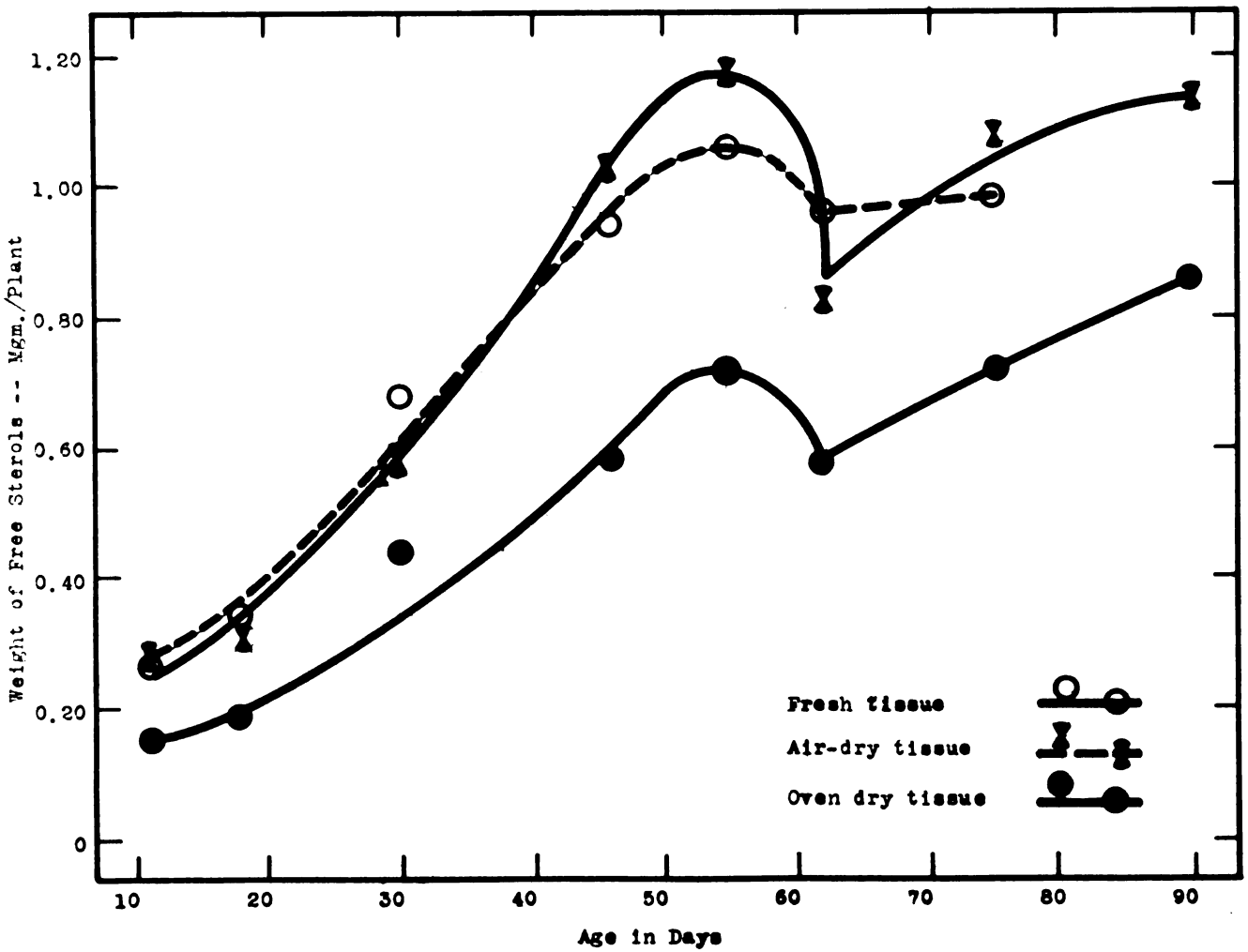
TABLE VII

FREE AND TOTAL STEROL CONTENT OF BEAN ROOTS

(1) % Lipid	(1) Mgm. Free Sterol per Gm. Tissue	(1) Mgm. Total Sterol per Gm. Tissue
2.18	.358	.724
2.22	.345	.699
	.350	.749
	.350	.764

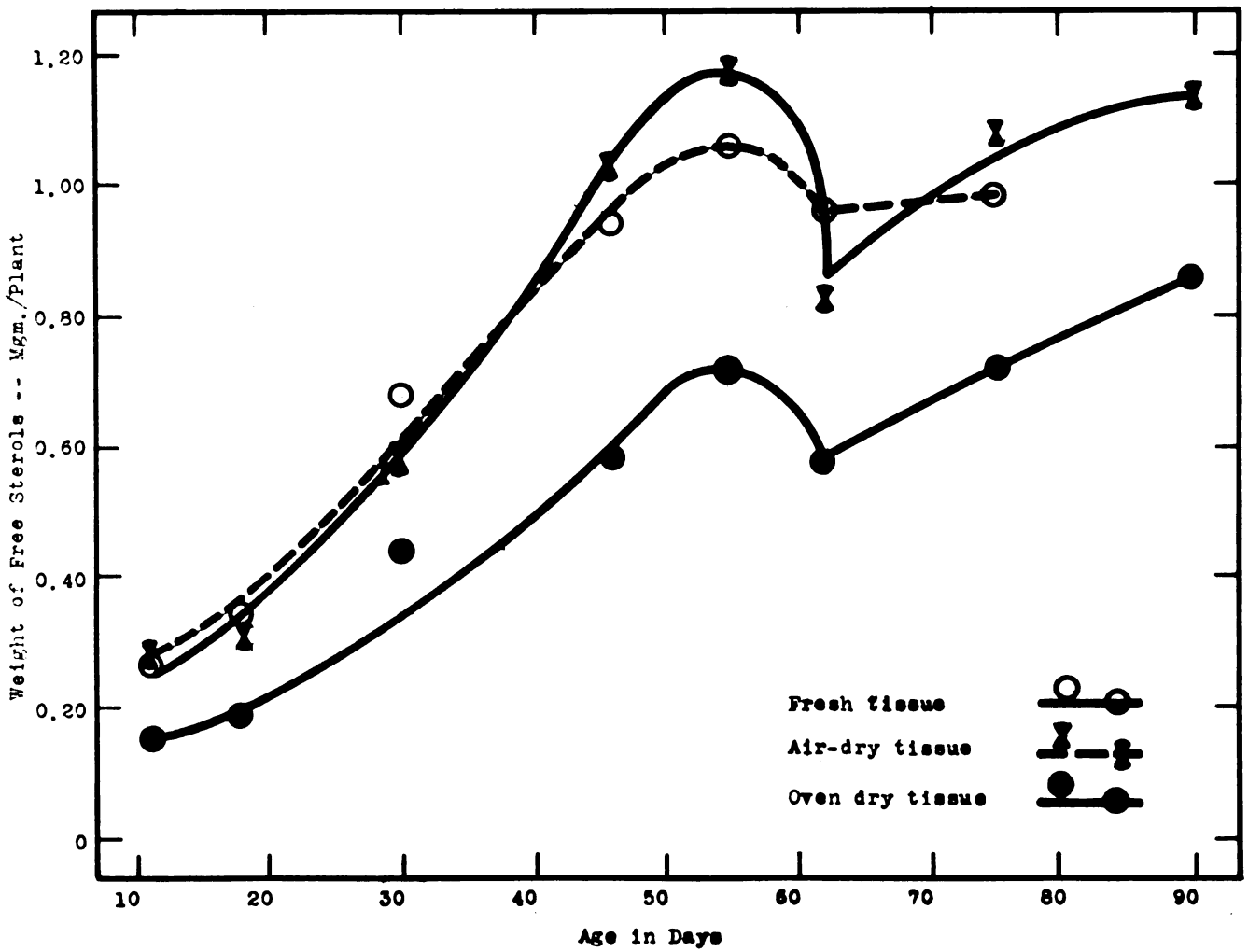
(1) Oven dry tissue

FIG. 6



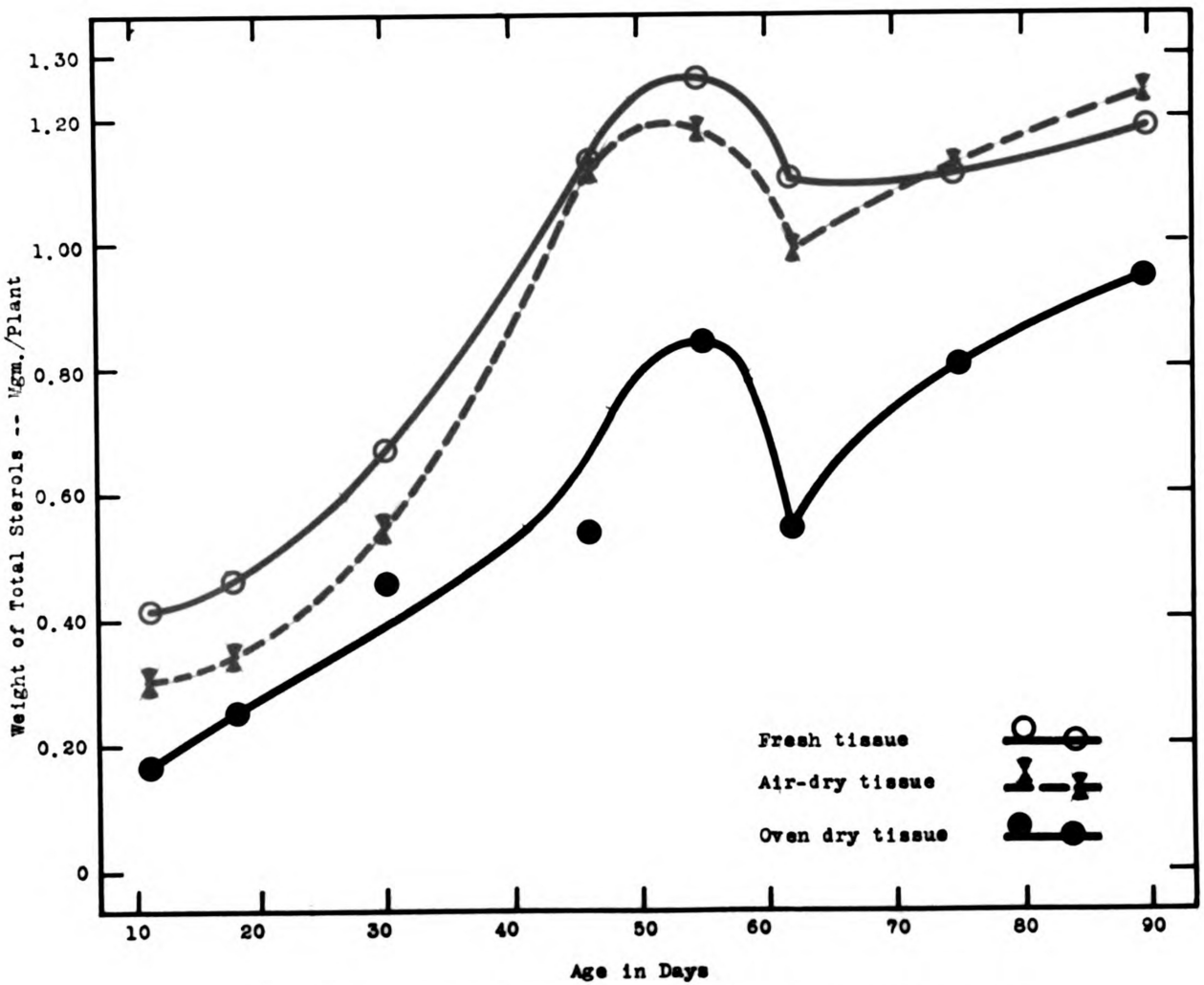
DEVELOPMENT OF FREE STEROLS WITH AGE IN PLANT, WITHOUT BEANS

FIG. 6



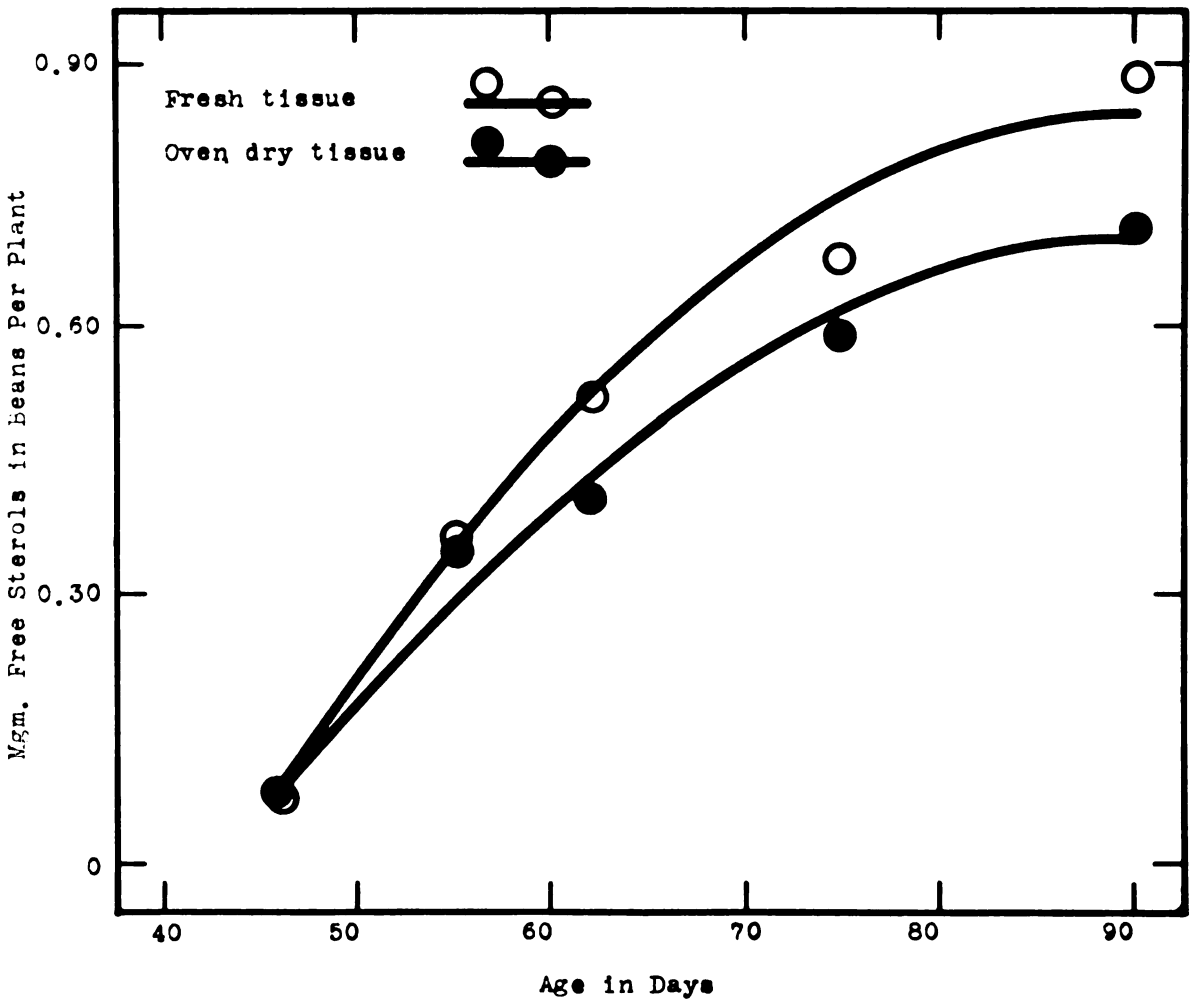
DEVELOPMENT OF FREE STEROLS WITH AGE IN PLANT, WITHOUT BEANS

FIG. 7



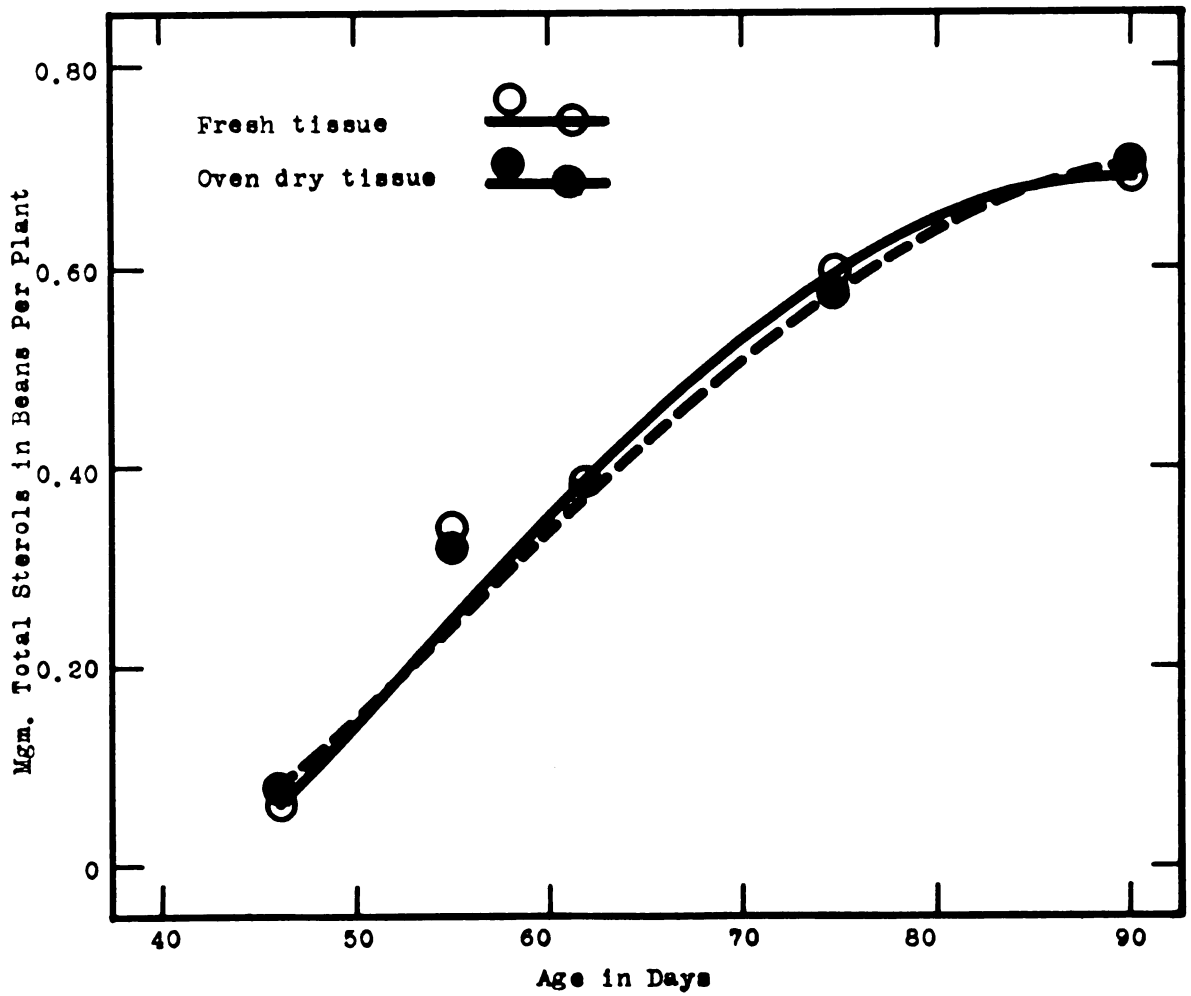
DEVELOPMENT OF TOTAL STEROLS WITH AGE IN PLANT, WITHOUT BEANS

FIG. 8



DEVELOPMENT OF FREE STEROLS IN BEANS

FIG. 9



DEVELOPMENT OF TOTAL STEROLS IN BEANS

V. SUMMARY AND CONCLUSIONS

1. Neither the plant nor bean tissue contained appreciable combined sterols.

2. Either the bean roots or the nodules on the roots or both contained combined sterols as well as free sterols.

3. The results for the sterol analysis of the fresh bean plant tissue and that which has been previously air dried, during all stages of development, are similar, but some destruction of sterols in oven dry tissue is apparent.

4. The results for the sterol analysis of fresh bean tissue and that which has been oven dried are similar, indicating that the beans may contain different sterols from those in the plant.

5. The sterol content of the bean plants increased rapidly up to about the 55th day of growth, after which it decreased until between the 60th and 65th day of growth, after which it increased again to around the level obtained about the 55th day. During this decrease in the sterol content of the plant, there is a rapid increase in the sterol content of the bean.

6. The sterol content of the beans increased continuously but the most rapid increase was during the first two weeks of growth of the bean.

7. The sterol development of the beans parallels that of the lipid development in that the most rapid formation is during about the first two weeks of growth, and shows a slow but continuous development to reach a maximum in the mature bean.

8. The lipid content of the plant tissue increased up to between the 50th and 55th day of growth, after which there was a decrease and later a slight increase.

9. It would appear that the bean plant containing beans can synthesize sterols at any stage of development since the increase in the sterol content of the beans more than accounts for the decrease in the sterol content of the plant.

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