

A NUTRITIVE STUDY OF VIGNA SINENSIS  
(BLACK-EYED PEA VARIETY)

BY

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# A NUTRITIVE STUDY OF VIGNA SINENSIS (BLACK-EYED PEA VARIETY)

## I. INTRODUCTION

For many centuries the blackeyed pea has been cultivated for domestic uses. It is a legume that will flourish where other crops fail, and even on poor soil it can be sown broadcast with reasonable expectancy of good growth. When planted on good soil and properly cultivated it produces a crop that has long been recognized as a foodstuff for man and animals, and as a green manure for enriching the soil on which it is grown.

Since in this country no extensive study of the food value of this material has been made, especially with regard to that portion of the material consumed by man, this project was initiated. The blackeyed variety was chosen for this work since it is considered to be the most palatable and because it is cultivated in this country as a foodstuff for man.

## II. HISTORICAL

Most authorities (Morse, 1919, Cates, 1919, Piper, 1913, Herman, 1919) agree that the cowpea is a native of Central Africa and that it has spread from there throughout a large portion of the civilized world. It was cultivated in ancient times for human food chiefly in Africa, Asia, and also in the Mediterranean countries of Europe. It was

brought to this country from the last named region early in the eighteenth century, being reported as cultivated in North Carolina in 1714. It has been stated by Carver (1917) that Oglethorpe introduced it into Georgia when he founded this colony in 1732. Thomas Jefferson<sup>1</sup> writes of its culture in Virginia as early as 1775 and he, himself, was enthusiastic about its palatability as a food.

During Colonial times, and in the first half of the nineteenth century it was apparently grown mainly for human consumption. However, following the Civil War it was a great boon to the south, as a means of recovering the fertility of the soil, lost as a result of previous agricultural practices. In fact, the reconstruction days might well be called the "Cowpea Era" (Cates 1919) of southern agriculture. As late as 1920 it was the best known and most extensively cultivated leguminous crop in the Southern States, but during the past fifteen years the soybean has more or less usurped this position.

According to Bailey (1935) there are three species of *Vigna*: (1) *Sinensis* (American Cowpea); (2) *Catjang Walp* (Indian Cowpea); (3) *Sesquipedalis* (asparagus bean). Each of these species represents a group of varieties having much in common and related to each other through intermediate varieties. The nomenclature of the cultivated varieties of

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1. The writings of Thomas Jefferson, Monticello edition, V.10, p. 12, Washington, D. C. 1904.



the cowpea and catjang is hopelessly confused, Piper (1913) enumerating 220 agricultural varieties of the former and 50 of the latter.

The name "blackeye" is a group name classed under the cowpea, applying in general to all white types with a blackeye. In some cases the varieties of this group can be satisfactorily identified. Extra Early Blackeye and Virginia Blackeye are two forms which are particularly adapted to this region. The California Blackeye is grown quite extensively throughout the interior valleys of the state from which it receives its name, and only recently a strong wilt resistant strain of this variety has been developed. (Whitney 1936).

The blackeyed cowpea may be prepared as a food in many ways. It is frequently eaten as a green vegetable, either in the pod or shelled. However, the pea is mostly eaten after it has been shelled and dried, in which case it is prepared in a similar manner to navy beans. In the old south it is especially well liked as a food, and has even been used to prepare such widely differing dishes as croquettes, pea soup, custard pie and cowpea coffee.

#### Historical Chemical

In the literature, very few references are found which deal with the chemical constitution of the cowpea. In general, the usual values for protein, fat, carbohydrate, fibre, ash and moisture on various portions of the plant are all the

data that are given. There may be found in Table I a compilation of these various values, all calculated to 10% moisture for purposes of comparison. In Table II may be found a compilation of the values reported for mineral constituents.

### Protein

The protein of the cowpea has perhaps been more widely studied than any other constituent of the plant, especially by workers in India, China and to a certain extent in Africa. However, most of these studies are not directly applicable from a nutritive standpoint as they are mainly concerned with the properties of the individual proteins and do not give an incite to the reactions of the total mixture of proteins present.

Osborne and Campbell (1897) first studied the proteins of the cowpea (*Vigna Catjang*) using a variety with a black seed coat, they separated a globulin which they named Vignin, a globulin which was similar to Phaseolin from the kidney bean, and a third globulin of unknown status. Osborne and Harris (1903) tried more or less unsuccessfully to identify the constituents of these separated proteins. However, Osborne and Heyl (1908) were successful in obtaining a fairly complete amino acid analyses of the Vignin. Osborne and Mendel (1912) reported experiments on feeding Vignin as the main protein constituent of the diet. Their results indicated that this protein was

satisfactory for maintenance, but was able to produce only very slight growth. Brewster and Olsberg (1919) reported a determination of the amino acids and nitrogen distribution in the whole pea and compared them with the values for corn and wheat. Fink, Jones and Johns (1922) reported the growth of rats on cooked and uncooked cowpea meal (Groit and Brabham variety) with and without a supplement of cystine. These workers found that only slight growth was obtained when the meal was uncooked, but that when it was cooked and supplemented with cystine normal growth ensued. They believed that the differences in results between cooked and uncooked meal could be accounted for entirely on the basis of differences in digestibility.

Much work has been done on the cowpea protein during the past five years. Niyogi, Marayana and Desai (1933) separated the globulins of *Vigna Catjang* and determined the amino acid content of them. They also determined the digestibility and the biological values on the whole pea (including the seed coat) at 10% level of protein intake, using rats as experimental animals. However, these last two values were obtained only on the cooked meal and furthermore the daily intake of brewer's yeast was not taken into consideration <sup>in</sup> the calculations.

Bhagvat (1935) prepared the total globulins of the cowpea (*Vigna Catjang Walp*) by four different methods and found these preparations could be separated into five definite

fractions by heat coagulation, and three definite fractions by  $(\text{NH}_4)_2\text{SO}_4$  precipitation. They reported the amino acid distribution on all fractions prepared and conclude that these fractions were different proteins, not only because of differences in amino acid content but also because of changes in optical rotation of the residual solution after removal of one or more of the fractions.

Adolph and Hsien-Ching Chiang (1935) separated the proteins of the cowpea (*Vigna sesquipedalis*) and found them to contain 45% Vignin and two other globulins, 5 and 10% respectively, as well as an albumin 15% and a glutelin 25%. They reported the nitrogen fractionation of each of these preparations.

In Table III may be found the values reported in the literature for the amino acid composition of the cowpea proteins.

#### Carbohydrate and Fat

So far as the writer can find there has been reported no detailed study of the carbohydrates or fats in this material.

#### Vitamins

Some work has been reported on the vitamin content of the cowpea. Indian Cowpeas (dried and powdered) present to the extent of 3% in the diet, were found by Jansen and Donth (1924) to be sufficient for a cure of avitaminosis A. Hermano (1930) claimed 5% of the green cowpea pods in the diet were

necessary for recovery from avitaminosis A. Fraps (1933), using small groups of rats found three Vitamin A units per gram of Blackeyed peas and that after seven, nine and fourteen months of storage 30, 50 and 75% respectively of the Vitamin A content was lost from the pea.

Goldberger and Wheeler (1927) proved that there was some pellegra-preventative factor in the cowpea by feeding experiments on twenty-one insane patients who had previous histories of pellegra.

Acuna (1923) found that five grams of fresh cowpeas (*Vigna Sinensis*) were necessary to promote normal growth in rats suffering from a lack of the Vitamin B complex.

Bankston and Giddings (1934) using blackeyed peas, obtained from a Louisiana feed store, found a value of 1.4 Sherman units per gram of Vitamin G or the lactoflavin factor.

TABLE I

## Alimentary Analyses of the Cowpea

Reported by	Country	Variety	Moist- ure	Fat	Fibre	Ash	Protein N x 6.25	N free Extract	Moisture as Reported
University <sup>1</sup> of Maryland:	United States	:Black- :eye pea :	: 10% :	:1.79 :	:3.63 :	:2.97 :	: 25. :	: - :	: 11.41 :
New York State(1916):	"	: not : given :	: - :	:1. :	: - :	:3.4 :	: 19.4 :	: 54.5 :	: - :
Webster (1928)	"	: " :	: 10% :	:1.53 :	: - :	:2.99 :	: - :	: - :	: 8.84 :
Mitchell & Mattison (1933)	"	: " :	: 10% :	:1.74 :	:5.02 :	:3.62 :	: 21.34 :	: 58.28 :	: 9.48 :
Bowers <sup>2</sup> (1919)	"	: average : of group:	: - :	:1.4 :	: - :	: - :	: 21.4 :	: 60.8 :	: - :
Prudhomme (1922)	: Indo- : China	:Sinensis: :	: 10% :	:1.17 :	:8.11 :	:3.15 :	: 25.1 :	: 44.41 :	: 12.14 :
French (1932)	: Tanganyika: : territory:	: " :	: 0.0 :	: .95 :	:5.57 :	:3.32 :	: 24.49 :	: 65.67 :	: - :
van Rosen (1927)	: Dutch E. : Indies	: " :	: 10 :	:1.97 :	:4.87 :	:3.66 :	: 25.3 :	: 45.2 :	: 13.1 :
Bhagvat (1935)	: India	:Catjang : Walp :	: 10 :	:2.71 :	:2.67 :	:2.95 :	: 26.94 :	: 40.43 :	: 11.03 :
Niyogi, Narayana & Desai(1931):	: India	:Catjang :	: - :	:1.24 :	:4.38 :	:3.99 :	: 26.62 :	: 64.37 :	: - :

1 - Sample submitted to Maryland State Chemist for analysis 1933.

2 - Reported a fuel value of 15.5 per pound.

TABLE II

## Mineral Analyses of the Copper

	Mitchell & Mattison <sup>2</sup> (1933)	Frenck <sup>2</sup> (1932)	van Rosen <sup>1</sup> (1927)	Bhagvat <sup>2</sup> (1935)	Bowers (1919)	Webster <sup>1</sup> (1928)
P					.532	.4736
P <sub>2</sub> O <sub>5</sub>	.99	1.49	1.2	.93		
CaO	.1275	.66	0.1	.22	.117 <sup>3</sup>	
MgO	.4037		0.3		.243 <sup>4</sup>	
Mn <sub>3</sub> O <sub>4</sub>	.0056					
Fe <sub>2</sub> O <sub>3</sub>	.0856		0.3	.013		
Al <sub>2</sub> O <sub>3</sub>	.0128					
S	.12				.280	
Cl	.05				.047	
N	3.44		4.5	4.16		
K <sub>2</sub> O			1.8		1.636 <sup>5</sup>	
Na <sub>2</sub> O			.01		.189 <sup>6</sup>	
SO <sub>3</sub>			.2			
SiO <sub>2</sub>		.21	.04	.05		
Cu				.004		

1. Calculated as moisture free basis

2. Calculated on air dry basis

3. Reported as Ca

4. Reported as Mg

5. " " as K

6. " " as Na

TABLE III

Amino Acid Distribution in Proteins  
Extracted from the Cowpea

	: Vignin <sup>1</sup>	: Whole <sup>2</sup> : Cowpea	: Total <sup>3,4</sup> : Globulins	: Vignin <sup>5</sup>	: Glutelin <sup>5</sup>	: Total : Globulins <sup>6</sup>	
Glycocoll	: .00	:	:	:	:	:	
Alanine	: .97	:	:	:	:	:	
Valine	: .34	:	:	:	:	:	
Leucine	: 7.82	:	:	:	:	:	
Proline	: 5.25	:	:	:	:	:	
Phenylalanine	: 5.27	:	:	:	:	:	
Aspartic Acid	: 3.97	:	:	:	:	:	
Glutamic Acid	: 16.89	:	:	:	:	:	
Tyrosine	: 2.26	:	: 3.81	:	:	: 2.67 - 4.80	
Cystine	: not det:	: 1.23	: .56	: 1.89	: 1.35	: 5.53	: .34 - 1.82
Arginine	: 7.20	: 17.63	: 15.2	: 7.7	: 7.54	: 6.33	: 10.09 - 13.43
Histidine	: 3.03	: 3.64	: 3.8	: 2.21	: 8.77	: 8.51	: 2.53 - 4.37
Lysine	: 4.28	: 5.93	: 9.1	: 5.96	: 11.41	: 3.89	: 5.31 - 7.39
Ammonia	: 2.32	:	:	:	:	:	
Tryptophane	: present:	:	: .7	:	:	:	: .51 - .59

All results, except those noted, expressed as percent total nitrogen in the material examined.

1. Reported by Osborne and Heyl (1908). Serine and Oxyproline not found.
2. Reported by Brewster and Alsberg (1919).
3. Reported by Miyagi, Narayana and Desai (1931).
4. Results expressed as percent ash and moisture free protein.
5. Reported by Adolph and Chiang (1935).
6. Reported by Bhagvat (1935).



### III. EXPERIMENTAL

As previously mentioned, the cowpeas used in this investigation were members of the blackeyed group. Four different lots were used during the course of the investigation. Lot One was obtained through a Washington seed store from some southern state, probably South Carolina or Georgia. Lot Two was obtained through a Baltimore seed house from the tidewater section of Virginia. Lots Three and Four were obtained through the University of Maryland Extension Service directly from the Eastern Shore of Maryland just after the crops were harvested. In general, the peas were stored at room temperature in burlap bags to simulate commercial conditions. For the determination of Vitamin A by direct feeding of the oil, a certain portion of Lot Three was stored at 0°C in order to protect the Vitamin A content.

For most of the experiments the peas were ground to pass a twenty-mesh sieve either by mechanical grinding in a coffee mill or by hand in a small corn mill. The whole pea was used, and in no feeding experiments was any attempt made to separate the pea into constituent parts.

#### Protein

The protein of the blackeyed pea was studied from a chemical standpoint only to the extent of determining the approximate isoelectric point of the total proteins extracted by water and sodium chloride, as well as the approximate quantities of nitrogen extracted by the usual protein solvents.

To determine the isoelectric point, samples of the meal were shaken with both distilled water and 10% sodium chloride in a mechanical shaker for an hour. The suspension obtained in this manner was centrifuged and the clear supernatant liquid was used in the determination. Each of these solutions was mixed with an equal amount of one normal sodium acetate and then treated with various increments of acetic acid in separate test tubes. Distilled water was then added to bring the volume of each solution up to 10 milliliters. A series of solutions were thus obtained which were observed for degree of precipitation. Finally, the pH was determined upon those solutions which showed the best precipitation. The results indicated that a pH range of 5.28 to 5.4 was best for precipitating the water soluble proteins while pH 3.97 - 4.89 was best for the 10% NaCl extractions.

Distribution studies were confined to the determination of total nitrogen extracted by various solvents from the petroleum ether free meal. These extractions were made with 70% alcohol, (Prolamines), 5% sodium chloride (Globulins), and .1% sodium hydroxide (Glutelins).

The 70% alcohol-soluble nitrogen was determined by the official method of the Association of Official Agricultural Chemists (1935)<sup>1</sup> for cereal foods.

The 5% sodium chloride-soluble nitrogen was determined by placing 5 gms. of petroleum ether extracted meal in a 50 cc. centrifuge bottle and adding 40 cc. of 5% sodium chloride.

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1. Chapter XX No. 17, Pg. 209.

The container was then corked and shaken in a continuous shaker for one hour, centrifuged fifteen minutes and the supernatant liquid transferred into a 250 cc. volumetric flask. The extraction was repeated four times. The extract was made up to volume with 5% NaCl, shaken, filtered through dry paper and a 100 cc. aliquot equal to two grams of the original material taken for the nitrogen determination.

The procedure was then repeated on the residual 5% salt extracted meal using .1% sodium hydroxide as the solvent. Blanks were run on the reagents. Nitrogen was determined by the Kjeldahl method.

The above determinations were made on Lot Two, containing 3.57% total nitrogen.

The results, expressed as percentage of the total nitrogen, are given in Table IV.

TABLE IV  
Nitrogen Distribution in the Blackeyed  
Pea

70% Ethyl Alcohol soluble nitrogen	5% NaCl soluble nitrogen	.1% NaOH soluble nitrogen	Residue by differ- ence
7%	70.3%	18.4%	4.3%

These values agree in the main with those reported by Bhagvat (1935) for Vigna Catjang Walp. This worker found 68% of the total nitrogen soluble in 5% sodium chloride, and 23.45% soluble in .1% sodium hydroxide. However, he found

only 1.2% nitrogen extracted by 70% ethyl alcohol as compared with our value of 7% for this fraction.

### Carbohydrates

In all the literature on cowpeas, no reference is made to any determination of the various types of carbohydrates present. An attempt was made, therefore, to find some of the more common values using the methods as outlined for grain and stock feeds in the A. O. A. C. Official Methods of Analyses (1935)<sup>1</sup>. Ten gram samples of meal were extracted with 50% alcohol, the alcohol removed, the resulting solution clarified with neutral lead acetate and excess lead removed with anhydrous sodium carbonate exactly as prescribed in the Official Method.

Ten milliliter aliquots of the resulting solution were subsequently tested for reducing sugar by the Scales Method (A.O.A.C. 1935)<sup>2</sup>, while other portions were hydrolyzed with hydrochloric acid and their reducing properties examined. The results of these determinations are given in Table V.

An indication of the biological availability of the blackeyed pea carbohydrate was obtained by studying the amount of reducing sugars produced by hydrolysis of the meal with taka-diaxase, followed by treatment with

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1. Chapter XXVII, No. 28-30, Pg. 341  
2. Chapter XXXIV, Nos. 34,35, Pg. 478

hydrochloric acid. The method used was essentially that proposed by Olmstead (1920) which includes the following steps: Cooking the meal, macerating the sediment, incubating for nineteen hours at 37°C in the presence of .1 gm. of taka-diastrase, using toluene as a preservative, clarification with neutral lead acetate, hydrolysis with hydrochloric acid, and determination of the reducing sugars by Scales Method. The results as shown in Table V were reported as starch. These values indicate that by "in vitro" methods the biological availability is approximately 52 or, in other words, that 52 % of the total carbohydrate is hydrolyzed by enzymes to simple sugars. This result is probably low compared with that to be expected with "in vivo" experiments for these conditions do not even approximately duplicate conditions in the animal body and taka-diastrase is an enzyme that does not hydrolyze the polysaccharides quite as completely as the body enzymes.

TABLE V

## Reducing Sugar and Sucrose in the Blackeyed Pea

	Reducing Sugar	Soluble carbo- hydrates report- ed as sucrose	Polyssaccharides hydrolyzed by taka-diestase reported as starch
	percent	percent	percent
Lot 3	.119	4.7	
	.119	4.9	
Lot 2	.119	4.0	31.2
Petroleum ether extracted	.146	3.9	30.9

Fat

A brief investigation of the characteristics of the oil obtained from the blackeyed pea was made<sup>1</sup>. The oil was obtained by petroleum ether extraction of the meal. The solvent was removed over steam and finally by desiccation over paraffin and paraffin oil. All analyses were made according to The Official Methods of Analysis of the A.O.A.C. (1932).

The extracted oil was slightly viscous, light yellow in color and possessed an odor similar to the cooked bean. The physical constants determined were: Specific gravity 0.915(25°/25°C) and refractive index 1.4727 (at 40°C.). The chemical determinations included: Saponification number 179.5 (mg/gm Koettstorfer number), soluble acids 1.11% (as butyric acid), insoluble acids 92.9% (Hehner number), soluble volatile acids 3.98 (Reichert-Meissel no.), insoluble volatile acids .55 (Polenske number), unseaponifiable residue 7.98%, Iodine number 89.1 (Hanus), Sat. Acids 23%, Unsat. Acids 57.7%.

1. Experimental work done by W. B. Lanham.

## . BIOLOGICAL ASSAY

Preliminary Experiments

In order to obtain some indication of the nutritive properties of the blackeyed pea, the following diets were fed to groups of two rats each and their reactions, amount of food eaten and amount of growth as evidenced by change in weight noted.

Diet (1) Stock Diet (see Materials Used).

(2) Blackeyed Pea alone.

(4) Blackeyed Pea 96 parts + salt mixture  
(McCollum's No. 185) 4 parts.

(5) Blackeyed Pea 95 parts + butterfat 5 parts.

(6) Blackeyed Pea 91 parts + butterfat 5 parts  
+ salt mixture 4 parts.

At the end of forty-five days each diet, except number one, was changed by the addition of five parts crude casein in place of an equal amount of the blackeyed pea, to determine the effect of supplementing the rations with a protein material of high biological value. The diets containing casein are characterized by the letter "A". Finally, on the seventy-second day the diets were again altered by the addition<sup>of</sup> either 10 parts dextrin and 10 parts lard or 8 parts yeast in place of equal amounts of the pea meal, while one of the rats on Diet 2A and one on 6A was given the stock diet.

The lard and dextrin were added to determine the effect of increasing the energy content of the diet while the yeast

supplement was supplied to determine the effect of increasing the Vitamin B-complex of the diet as well as the protein content.

The results are charted in Graph One. This chart indicates that the blackeyed pea when fed alone (Diet 2) would maintain life and promote a very small growth in the animal for periods up to forty days, but thereafter a gradual decrease in weight resulted even though on the forty-fifth day the diet was supplemented by such an all-round protein material as casein. In view of the fact that most dietary deficiencies do not immediately show their effects on the animal the more or less flat growth curve found in the early stages points to either protein deficiency or a lack of Vitamin G. By the forty-fifth day of an experiment the lack of almost any important item of diet would make itself felt and therefore the absence of effect when casein was added to the diet (Diet 2-A) merely shows that this protein was not the only food factor present in insufficient quantity. By the seventy-second day of the experiment the rats on this experiment developed a rachitic gait, and at the same time had a tendency to lose a lot of hair, becoming quite bald in spots. One of the rats was therefore given a supplement of 8% yeast to determine if the presence of large quantities of Vitamin G and of sufficient cystine would restore a normal fur. A slight gain in weight did result for about ten days, which would indicate that some of the deficiencies of the pea are found



in yeast. However, this addition was still not enough to supplement all the factors lacking in the diet of this rat, for following the initial gain he lost weight rapidly and died twenty days after the change. The fact that neither yeast nor casein were able to supplement this diet would point to a major deficiency of the fat soluble vitamins in the blackeyed pea.

The other rat on diet 2-A was placed on the stock diet to see if such a long period on the pea meal alone had impaired any of the growth faculties of the animal. This animal immediately began to grow, and the growth was at a normal rate paralleling closely the curve for rats on the stock diet. At the same time his hair began to grow again and at the end of thirty-five days after the change he was apparently perfectly normal. His diet was therefore again changed to the blackeyed pea meal alone (Diet 2). This resulted in an abrupt decrease in rate of growth but some increase in weight was noted for a period of eighteen days. This was followed by a period of seventeen days during which the weight was practically constant. After this period a decline in weight resulted but the animal lived for seventy-five days after being returned to the restricted diet.

Rats receiving blackeyed pea plus salt mixture (Diet 4) during the first ten days showed gains equal to more complete diets, and then remained at practically constant weight for the duration of the experiment. One

rat remained when the diets were supplemented with casein (Diet 4-A) and although he gained a slight amount he died within nineteen days after the change in diet. Consistently more of the salt supplemented diet was eaten than that of the pure blackeyed pea which indicates that it was more palatable. Comparatively large amounts of it were eaten at the beginning of the experiment which no doubt accounts for early gains of this group. The diet did not prove to be much better than the blackeyed pea alone; even the increased food consumption did not cause enough difference to prove of lasting effect. Finally both rats on this diet died by the sixty-fourth day of the experiment.

When the blackeyed pea meal was supplemented with butterfat (Diet 5) better growth was obtained and more food was eaten. This continued for the first forty-five days. From the forty-fifth to the seventy-second day even though casein (Diet 5-A) was added to the diet the growth increase was only slight. After the seventy-second day one of the two rats on this diet was fed a yeast supplement while the other received lard and dextrin. The former showed immediate improvement not only in growth but in appetite, although he did not return to normal. The other rat merely maintained its weight.

When the pea meal was supplemented with both butterfat and salt mixture (Diet 6), although normal growth was not obtained, improvement in growth and consumption was noted,

compared with the previously mentioned diets. The growth was only slightly greater than for the rats receiving the meal plus butterfat during the first period of the experiment. Five rats received this diet, all of them lived and when given the casein addition (Diet 6-A) showed a decided increase in growth, indicating their limiting deficiency was protein in nature. On the seventy-fifth day this set was divided into three sub-groups. One rat was placed on the stock diet, two received an 8% yeast supplement and two received 10% lard and 10% dextrin. The rat receiving the stock diet immediately showed a normal rate of growth. Those receiving dextrin and lard exhibited continued growth, but at no faster rate than without the supplement. Those receiving the yeast showed a notable increase in weight but their growth was not normal.

These preliminary experiments seem to point to the following conclusions: (1) The blackeyed pea meal as sole dietary material will maintain life for periods of at least forty-five days after which the addition of casein makes only slight, if any, improvement. (2) Addition of a complete mixture of inorganic salts or butterfat produces only slightly better results, the latter addition being more effective than the former indicating that the fat-soluble vitamin content and the salt content of the black-eyed pea are not sufficient for normal growth. (3) The addition of large quantities of energy producing materials such as dextrin and lard did not, in any instance, show

improvement, indicating that the energy content of the pea meal is not a primary limiting factor. (4) The addition of yeast and casein to the diets generally produces improvement, indicating that either the Vitamin B complex or protein content is a limiting factor. (5) At the end of long periods on restricted diets the animals are still able to gain normally if put on a complete diet.

### Protein Studies

To determine more accurately the value of the black-eyed pea, nitrogen for the maintenance and growth of rats, diets were prepared (Table VI) containing ten, fifteen, and eighteen percent of the pea protein. These were fed to three groups of nine rats as well as to rats receiving no protein, and to rats receiving a mixture of proteins from wheat flour middling and casein. This last diet was prepared so as to be complete in all respects.

TABLE VI

**Blackeyed Pea Protein Diets for Study  
of Maintenance and Growth**

	D-11	D-11A	D-12	D-13	D-14	D-15	D-18
Salt Mixture	4	4	4	4	4	4	4
Butterfat	8	8	8	8	8	8	8
Sodium Chloride	1	1	1	1	1	1	1
Yeast	2	2	2	2	2	2	2
Cod-Liver Oil	2	2	2	2	2	2	2
Dextrin	73	-	32.5	12	-	18	-
Sucrose	10	-	10	10	4.8	10	4.7
Blackeyed Pea	-	83	40.5	61	73.2	-	78.3
		(lot 1)	(lot 1)	(lot 1)			(lot 2)
Wheat flour middlings						45	
Casein						10	
Percent Protein	0	20.5	10.0	15.0	18.0	18.0	18

These results are indicated in Graph Two. As was to be expected, rats on the protein free diet rapidly lost weight. When it was thought impossible for them to live longer on this diet the various carbohydrate materials present were replaced with the blackeyed pea meal and an immediate and rapid growth resulted, which for the space of fourteen days was almost equal to that of normal rats. This rapid rate of growth was followed by a gradually decreasing rate of growth until it assumed proportions similar to the groups receiving the higher percentages of pea meal.

The group receiving only 10% protein gradually lost weight and all but two were dead by the forty-first day, showing that the pea protein when supplied at this level is not sufficient

even for maintenance. Rats receiving 15% and 18% pea protein continued to gain weight for from fifty to sixty days but thereafter the protein evidently was not sufficient to support further increase in growth, as the curves level off almost completely. The rats were observed for ninety-six days (during which time two in each group died) but, although their food intake held up fairly well, a maximum growth was not maintained. It is interesting to note that for the first fifty days the food intake per rat per day for both groups averaged exactly the same. (Table VII). However, the diet of greater nitrogen content produced more growth during this period and the increase in weight per gram of nitrogen consumed was also greater.

TABLE VII

Food Consumption and Growth on Blackeyed  
Pea Diets

	Food Consumed per rat per day	Average growth 51 days	Protein Con- sumed	Av. Growth per rat per day	Av. Growth per gram protein eaten
D-13	5.15	22.6	.7725	.443	.573
D-14	5.15	31.5	.927	.618	.866

Cystine Supplements

The effects of cooking the blackeyed pea meal as well as supplementing it with cystine were next studied. The basal ration used in this experiment is given as Diet 18

in Table VI. Four variations of this diet (D-18, D-19, D-20, D-21) were fed to groups of eight rats each. Diet 20 is similar to Diet 18 but the former contains cooked blackeyed pea. Diets 19 and 21 are similar to Diets 18 and 20 respectively, but the former are supplemented with .36% cystine which equals 2% of the protein present. Feedings were continued for twenty-four days at which time the diets were changed so as to provide extra cystine for some of the rats. The pea meal was present in a sufficient amount to furnish 18% protein to the ration, and was cooked by making it into a paste with water and autoclaving it for three hours at fifteen pounds pressure.

The diets used in the first series of experiments contained 2% yeast, insuring ample amounts of the Vitamin B complex, for it was thought that this vitamin might be insufficient in the autoclaved meal. It is believed that the uncooked meal contains ample quantities of this food essential when taken in the amounts such as consumed during these tests. To see the affect of no yeast present in the diet, another similar set of four groups of three rats each were fed as above, but with this constituent replaced by an equal amount of sucrose. Also, the method of heat treatment for the protein was altered in that the paste was kept in a boiling water bath for three hours rather than autoclaved.

TABLE VIII

Food Consumption and Growth on Diets Containing Cooked Blackeyed  
Pea Meal and Cystine

Diets	Series I Containing Yeast : 24 - 61 days				Series II no Yeast : 0 - 42 days			
	Av. gain : in body : weight	Av. daily : food : intake	Av. gain : in body : weight	Av. daily : food : intake	Av. gain : in body : weight	Av. daily : food : intake	Av. gain : in body : weight	Av. daily : food : intake
Raw Pea D-18	33.3	6.83	1.134	34.0	7.57	33.7	5.76	.773
Raw Pea + .36% cystine D-19	39.0	6.62	1.361	62.8	8.81	42.7	5.48	1.031
Raw Pea + .72% cystine D-19-A				52.5	8.09	.976		
Cooked Pea D - 20	38.0	7.24	1.211	24.4	7.37	53.3	5.85	1.204
Cooked Pea + .36% Cystine D - 21	56.5	7.48	1.745	49.9	7.92	92.	7.15	1.704
Cooked Pea + .72% Cystine D-21-A				54.0	8.07	1.012		



From the results, which are reported<sup>1</sup> in Table VIII, a number of facts concerning the pea may be obtained. Autoclaved blackeyed pea meal produces greater growth in the young rat than does the raw pea meal. However, the rate of growth on the autoclaved meal markedly decreases as the rat becomes older. This effect is not found for the raw pea meal nor is it found when lower temperatures are used for cooking, pea meal cooked in a boiling water bath being in all instances superior to the raw meal, even when the latter is supplemented with cystine.

The addition of cystine to any diet containing only the blackeyed pea meal as a source of protein produces an increase in rate of growth. With very young rats the supplemented diet containing the untreated pea is not eaten as well as the unsupplemented diet, but in spite of this lower intake a greater growth results. As the rats grow older and the difference in average weight between the two groups becomes larger, it is natural that the heavier rats would consume more than the lighter ones, and this is evidenced by a greater increase in average daily consumption for the rats receiving the supplemented diet. The lower level of daily intake for the supplemented diet would seem to indicate that this diet is less palatable.

No such difference in daily intakes is noted when the pea in the diet has been heated. A greater daily food intake is noted for the cystine supplemented cooked pea diet regardless of the age of the rat or the method of heat treatment.

Rats receiving the raw pea supplemented with cystine grew approximately one and one-quarter times as much per gram of protein eaten as those not receiving cystine, while with the heat treated meals the increase in growth was one and one-half times as much for the cystine supplemented diet. When the cystine supplement was doubled for any of the above diets no further increase in rate of growth and no greater growth per gram of protein eaten resulted. None of these diets produced a normal rate of growth, and it is evident that there is some further or secondary amino acid deficiency in the blackeyed pea meal, which, while it is present in sufficient quantity to permit some growth, becomes the growth limiting factor when ample quantities of cystine are added to a diet containing the blackeyed pea as the sole source of protein but complete in all other respects.

#### Secondary Amino Acid Deficiency

An indication of the nature of the secondary amino acid deficiency was obtained by feeding the rats used in the previous experiment diets containing supplements of proteins of known amino-acid content.

Rats that had been receiving the above diets for eighty-one days were rearranged into five groups. Two groups received Diets 18 and 19 respectively, while the other three groups received Diet 18 with 5% of its black-eyed pea content replaced by an equal amount of casein,

(Diet-22) yeast (Diet-24), and gelatin (Diet-26) respectively. These materials are practically pure proteins and the effect of their addition was to increase the nitrogenous material in the diet by five percent and to alter the black-eyed pea content so that it furnished only 16.8% of protein to the diet.

The rats continued on these diets for twenty-nine days. The rats with the yeast in the diet ate the most food during this period, but those receiving casein produced the maximum growth increase. The rats receiving the plain pea supplemented with cystine ate more than those receiving the casein, but did not grow as much as those receiving the yeast. Rats with a gelatine supplement produced no more growth than those on the plain pea and in some instances lost weight. X

A consideration of these results leads to the conclusion that under the conditions of this experiment, gelatin is not any help as a supplement to the blackeyed pea protein but that casein is of decided benefit and yeast of some benefit.

These results would be expected if tryptophane or tyrosine was the secondary limiting deficiency, for reports that gelatin contains no tryptophane, and only a minor amount of tyrosine whereas the other two proteins, yeast (Csonka 1935) and casein, contain ample amounts of these amino acids. Most values reported for the content of these amino acids in the cowpea show a fair quantity of tyrosine but only slight quantities of tryptophane.

### Nitrogen Balance Studies

The bioassays thus far reported in this paper have been entirely concerned with food consumption and growth on diets varying only in total nitrogen present, or in amino acids present. This is one way of evaluating a protein. Another method is to feed it in small amounts as the sole source of nitrogen, except perhaps for vitamin supplements, and, by accurate analyses of faeces and urine, to calculate the amount absorbed as well as the amount utilized by the system. From this data there may be obtained the so-called digestibility coefficient and the biological value of the protein. These factors are represented by the following equations:

$$\text{Absorbed Nitrogen} = \text{N Intake} - (\text{Faecal N} - \text{Metabolic N in faeces})$$

$$\text{Utilized Nitrogen} = \text{Absorbed N} - (\text{Urinary N} - \text{Endogenous N in the urine})$$

$$\text{Digestibility} = \frac{\text{Absorbed Nitrogen}}{\text{Nitrogen Intake}}$$

$$\text{Biological Value} = \frac{\text{Utilized Nitrogen}}{\text{Absorbed Nitrogen}}$$

Both the metabolic nitrogen in the faeces and the endogenous nitrogen in the urine must be determined by feeding diets practically devoid of this element (so-called nitrogen-free diet). The urinary nitrogen excreted by rats receiving such a diet must result from the catabolism of body tissues and is called the endogenous nitrogen. This

value has been proved by Fixsen and Jackson (1932) to be practically constant for each rat, provided its weight exceeds one hundred and fifty grams. The faecal nitrogen is derived from the intestinal tract and its secretions and is called the metabolic nitrogen. According to Mitchell (1923) this is independent of the body weight of the rat but varies in proportion to the food consumption. It is necessary, therefore, to ascertain the faecal nitrogen excreted per gram of food on a nitrogen free diet and multiply this value by the grams of food consumed on any other diet to determine the total metabolic nitrogen for any particular test. This method of determining protein efficiency was proposed by Karl Thomas, but was brought to its present stage of development by Mitchell (1923).

The apparatus used in our experiments has been described by Lanham (1937) and consists in brief of an animal cage with a false bottom placed over a large paraffin coated metallic funnel. A fine mesh wire screen was placed near the apex of the funnel to retain the faeces while a jar containing fifty percent sulfuric acid was placed underneath to catch and preserve the urine.

The tests were run with a two-day preliminary period to allow the animals to become accustomed to their new diet, and at the same time to permit time for the dissipation of nitrogenous materials present in the intestine at the start of the experiment. This was followed by four days during which the faeces and urine were collected. An attempt was

made to keep the food consumed approximately uniform for each animal for the duration of the experiment, and at the same time prevent a gain or loss in body weight.

This was in some instances difficult to do, especially when the diet was very low in nitrogen. (Nitrogen-free diets). On these diets the animals refused to eat sufficient quantities of food and generally lost considerable weight. In order to decrease to a minimum the possibility of abnormal tissue breakdown, which generally follows loss of weight, the collection period for these tests was reduced to three days.

During the course of this experiment two different series of tests were attempted. In the first series, using rats "A" to "F", the protein was fed at 10% levels and ample quantities of the Vitamin B-complex were present, even in the nitrogen-free diet. In the second series<sup>1</sup>, using rats "A" to "L" the protein was fed at 5% levels and the nitrogen present as a vitamin supplement was greatly reduced. Series I consisted of four different test diets, three of which varied only in the method of treating the blackeyed pea meal. These three treatments were: (1) uncooked; (2) autoclaved; (3) heated on a boiling water bath. The fourth diet contained casein and was fed in the nature of a control, many comparative values for this protein being

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<sup>1</sup>Experimental work for series II done by Willis H. Baldwin.

published in the literature. Series II consisted of two test diets, one containing the raw pea meal, the other the autoclaved meal. These diets are given in the following table.

TABLE IX  
Metabolism Diets

	Protein at 10% level	Protein at 10% level	Protein at 5% level	Protein Free Diet I	Protein Free Diet II
Butter	8	8	8	8	8
Cod Liver Oil	2	2	2	2	2
Treated Dextrin	10	10	-	10	-
Salt Mixture (U.S.P.X. 1934)	4	4	4	4	4
Labco XXX	4	4	2	4	2
Dextrin	25.7	62	60.8	72	84
Blackeyed Pea Meal	46.3		23.2		
Casein		10			

The results are reported in Table X. The digestibility is found to be approximately seventy-eight, none of the averages varying enough from one another to be statistically significant, although the diet containing pea meal cooked in a water bath does seem to be slightly more digestible as indicated by a value of  $80 \pm .4$ .

The biological values differ with the method of heat treatment as well as the amount of blackeyed pea meal present. It is a well-known fact (Forbes et al, 1935, Boas-Fixsen 1935, Mitchell 1923) that an increase in the plane of protein intake results in diminished efficiency in the utilization of food nitrogen. A lower biological value for the 10% levels of feeding is therefore to be expected. This is found to be the case.

The cooked meal was found superior to the raw meal in every instance, but the biological value of the autoclaved meal is lower than that for meal which is heated at ordinary atmospheric pressure. Apparently, either the higher temperature or the higher pressure or both of these factors contributed to a changing or denaturing of the cowpea protein so that its efficiency is diminished.



Biological Value and Digestibility of Cooked and Uncooked Blackeyed Pea Protein

Non-Protein Diet I (.179% Nitrogen)

Ret	Initial Weight	Change in Weight	Daily Food Intake	Daily Nitrogen Intake	Daily Faecal Nitrogen	Daily Urinary Nitrogen	Total Nitrogen	Metabolic Nitrogen	Digestibility	Biological Value
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	%	Value
A	183	-4	11.3	20.2	31.2	7.1	2.75			
B	209	-10.5	9.86	17.6	26.2	33.1	2.65			
C	189	-8	9.33	16.7	21.3	39.9	2.28			
D	199	-3	13.83	24.8	30.9	37.7	2.23			
E	1835	-2	11.75	21	29.1	33.4	2.48			
F	1995	-9.5	6.66	11.9	25.3	23.4	3.78			

Cooked Blackeyed Pea Protein at 10% Level (1.85% Nitrogen)

(Lot 1 heated in a water bath)

A	222	+7	12.1	225	74.1	100.5	33.3	80.2	50.5
B	245	+5.5	14.	260	81.4	135.	37.1	82.8	52.7
C	215.5	+4.5	11.6	215	62.1	112.	26.4	83.4	60.1
D	236.5	+7.5	13.25	246	80.4	126	26.1	77.9	54.
E	231	+2.5	12.1	225	77.1	92.8	30.0	79.	66.5
F	233	+3.	10.6	197	65.1	94.1	26.0	80.2	55.2
Av.								80.6 ± .4	56.5 ± 1.7

TABLE X (CONT'D)

## Biological Value and Digestibility of Cooked and Uncooked Blackeyed Pea Protein (Cont'd.)

Cooked Blackeyed Pea Protein at 10% Level (1.81% Nitrogen)  
(Lot 2 Autoclaved)

Rat	Initial Weight	Change in Weight	Daily Food Intake	Daily Nitrogen Intake	Daily Fecal Nitrogen	Daily Urinary Nitrogen	Total Metabolic Nitrogen	Digestibility	Biological Value
	gm.	gm.	gm.	mg.	mg.	mg.	mg.	%	Value
B	258	-8	11	199	61.5	107.4	29.1	83.7	50.2
C	219.5	-2	10.25	186	61.8	112.3	23.4	79.3	50.9
D	268.	-4	11.5	208	78.6	114	25.6	74.6	48.
E	237	-8	10.37	183	68.4	109.4	25.7	77.2	53.2
F	238	-7	9.	163	70	106.2	23.4	71.4	61.3
AV.									77.2 ± 1.4: 52.7 ± 1.5

## Raw Blackeyed Pea Protein at 10% Level (1.81% Nitrogen)

(Lot 2)

A	241	0	11.5	208	78.8	106.2	31.6	76.6	38.4
B	266.5	-2	13.56	245	78.7	126	35.9	82.5	54.3
C	227	0	11.6	210	67.	120	26.4	80.7	47.6
D	264	+2	11.75	213	86.1	112	26.2	71.5	46.3
E	246	-1	11.75	213	74.9	99.6	29.1	78.5	47.
F	241	+2	11.75	213	77.2	113.	30.5	78.4	47.
AV.									78 ± 1 : 46.8 ± 1.1

TABLE X (CONT'D.)

## Biological Value and Digestibility of Cooked and Uncooked Blankeyed Pea Protein

## Non-Protein Diet II (.135% Nitrogen)

Ret	Initial Weight	Final Weight	Change in Weight	Daily Food Intake (gm.)	Daily Nitrogen Intake (mg.)	Daily Faecal Nitrogen (mg.)	Daily Urinary Nitrogen (mg.)	Total Nitrogen (mg.)	Metabolic Nitrogen (mg.)	Digestibility	Biological Value
G	204	204	-5	12.3	16.6	33.6	34.8	2.73			
H	204	204	-5	7.2	9.72	15.0	26.3	2.09			
I	227	227	-5	8.8	11.9	22.2	32.1	3.52			
J	214	214	-4	7.7	10.4	15.9	26.8	2.09			
K	215	215	-8	8.7	11.7	15.3	26.8	1.76			
L	205	205	-8	8.7	11.7	18.3	29.6	2.10			

## Cooked Blankeyed Pea Protein at 5% Level (.94% Nitrogen)

## (Lot 2 Autoclaved)

G	201	201	-3	13.4	126	54.5	59	36.6	85.8	77.6
H	202	202	+1	13.4	126	56	46.3	28	77.7	79.7
I	234	234	-3	10.6	99	44.3	53	37.3	93	77.2
J	214	214	0	10.6	99	52.5	47	21.9	69.1	70.4
K	216	216	0	8.0	75	40	48	14.4	65.8	57.1
L	211	211	-9	10.1	95	35.8	50	21.2	84.5	74.6

AV. : 79.3 ± 3.2; 72.8 ± 2.2

TABLE X (CONT'D.)

Biological Value and Digestibility of Cooked and Uncooked Blackeyed Pea Protein (Cont'd.)

Raw Blackeyed Pea Protein at 5% Level (.93% Nitrogen)  
(Lot 2)

Rat	Initial Weight	Change in Weight	Daily Food Intake	Daily Nitrogen Intake	Daily Faecal Nitrogen	Daily Urinary Nitrogen	Daily Faecal Nitrogen / gm. of food	Total Metabolic Nitrogen	Digestibility	Biological Value
	Gm.	Gm.	Gm.	Mg.	Mg.	Mg.	Mg.	Mg.	%	
O	212	+6	13.6	127	65.3	65.5	65.5	37.1	77.8	69
H	217	+2	13.4	125	57.5	66.0	66.0	28.0	76.5	57.7
I	241	-2	12	112	49.3	54.5	54.5	42.3	93.8	78.6
J	229	+3	12	112	54.8	56.5	56.5	24.9	73.3	64.
K	227	+2	11.1	103	47.3	52.8	52.8	19.6	73.2	65.5
L	220	+1	12.9	120	55.3	59.8	59.8	27.1	76.5	67.2

AV. : 78.5 ± 1.9; 67.0 ± 1.7

Casein at 10% Level (1.45% Nitrogen)

A	203	+7.5	12.7	165	45.8	42.4	42.4	34.9	94.1	80
B	230	+2.5	14.2	207	47.5	73.7	73.7	37.6	95.1	79.4
C	200	+5	12.2	178	37.3	73.3	73.3	27.8	94.4	80.4
D	216	+9	13.6	197	42.1	40.1	40.1	30.3	94.3	72.2
E	215	+8	12.6	183	36.8	83.	83.	31.2	96.9	72.2
F	217	+6.5	12.7	184	40.1	60.1	60.1	30.	94.5	79.

AV. : 94.9 ± .3; 78.2 ± .8

### Vitamin A Determination

The method of assay used was similar to that described by Lee and Tolle (1934).

The diet used was as follows:

Casein (Vitamin Free)	18%
Salt Mixture (U.S.P.X.No.1)	4
Agar	2
Yeast	7.5
Yeast Irradiated	0.5
Dextrin	66.
Cottonseed Oil	<u>2</u>
	100%

Several litters of rats were obtained from the Sunny Hill Rabbit Farm of Washington at seventeen days of age while still with the mother, and immediately put on the Vitamin A Free diet. Between twenty-four to twenty-eight days of age, and when they had obtained a weight between forty to fifty grams the rats were weaned and placed three to a cage with the diet fed ad libitum. At the end of twenty-four to twenty-eight days the rats had reached a constant weight and many of them showed signs of xerophthalmia. They were then separated into groups so that all groups were as nearly similar as possible with respect to weight, sex, age and litter.

Two methods were attempted for determining the amount of Vitamin A in the blackeyed pea. (1) The feeding of daily supplements of blackeyed pea meal; (2) The feeding of extracted blackeyed pea oil in various dilutions with cottonseed oil.

Method One - Five groups of rats were chosen. One group received no supplement and acted as a negative control while another received a daily supplement of one-tenth of a milliliter of a cottonseed oil solution containing three International Units of Vitamin A. This solution was prepared from the United States Pharmacopoeia Standard Reference oil. The other three groups received 0.5, 0.75 and 1.0 gm. daily of blackeyed peas respectively.

After twenty-eight days on test the control rats showed an average gain of 41.5 gms. which is the result to be expected from a daily intake of three units of Vitamin A. None of the rats receiving blackeyed pea supplements showed any gain so that no calculations of the amount of Vitamin A present were possible. However, the negative controls died in shorter period of time and lost weight more rapidly than any of the test animals, and of the test groups, those receiving the larger amounts of pea ration lived longer and lost weight less rapidly than those receiving smaller amounts. While this test proves nothing from a quantitative standpoint, it does indicate that there must be some Vitamin A in the blackeyed pea, otherwise the test groups would have paralleled the negative controls of each other.

Method Two - In addition to negative and positive control groups, three groups of rats were used and given a 0.1 cc of the following oils as a daily supplement. No. 1 - 1 gm.

blackeyed pea oil made up to 10 cc with cottonseed oil; No. 2 - 5 gms. of pea oil made up to 10 cc with cottonseed oil; No. 3 - pure blackeyed pea oil.

The blackeyed pea oil was obtained by extracting the freshly ground meal with petroleum ether. The peas used were purchased directly from the grower (Eastern Shore of Maryland) and on arrival were placed in a friction top can in the hardening room in the University Dairy Building. The temperature of this room is approximately 0°F. The peas were stored there throughout the duration of the test and small lots removed as needed every week. On removal from the cold storage, the peas were immediately roughly ground and extracted with petroleum ether in a large continuous extractor for two days. This removed most of the water which prevented thorough grinding originally. The coarse meal was then removed from the extractor and ground as fine as possible in a corn mill. On further extraction the last traces of oil were removed. The various extracts were then combined and the solvent recovered by distillation, first on a steam bath down to 300 cc. of residue and then under reduced pressure down to a volume of 30 cc. Finally the last traces of solvent were removed by use of a high vacuum in conjunction with a continuous stream of CO<sub>2</sub>. This was continued until a constant refractive index resulted.

The oil was prepared fresh every week as were the

solutions of oil which were fed to the rats. These latter were made up by weighing out the required amount of oil and then adding the calculated volume of cottonseed oil to make 10 cc. The oil was used exactly as it was prepared without further purification or separation of the solid constituents, so that as little possible oxidation of the Vitamin A in the oil might take place.

The test was continued for sixty days, and the results as presented in Graph Four, indicate that the blackeyed pea oil has approximately 2.9 International Units of Vitamin A per gram, or that there are 0.4 International Units of Vitamin A per gram of blackeyed peas.

#### Vitamin D Determination

The method for the determination of Vitamin D was carried out as described in the United States Pharmacopoeia (1934).

The diet used was No. 2.

Whole Yellow Maize, ground	76%
Ground Gluten	20%
Calcium Carbonate ( $\text{CaCO}_3$ )	3%
Sodium Chloride ( $\text{NaCl}$ )	1%
	<hr/>
	100%

Rats from the Sunny Hill Rabbit Farm weighing between 55 - 62 gms. were placed three in a cage and fed the Vitamin D deficient diet. This diet was continued for from twenty-one to twenty-five days, or until evidence of rickets was manifested



by a distinctive wobbly gait, and by enlarged joints. In addition, at the end of twenty and twenty-one days respectively, a rat was killed and analyzed for rickets by the line test. The rat on diet for twenty days showed incipient rickets while that killed on the twenty-first day was definitely rachitic by the line test.

When rats were definitely rachitic they were placed in separate cages and fed the rachitogenic diet, and distilled water ad libitum. Throughout the first eight days of the assay period each rat was fed an appropriate dose of oil depending upon the group in which he was placed. On the ninth and tenth day no oil was fed any rats, and on the eleventh day of the assay period each rat was killed and both forelegs removed and cleaned of fur and excess tissue.

Healing of the rachitic metaphysis was determined by use of the line test as follows: the distal end of a radius or ulna was thoroughly cleaned of adhering tissue and a longitudinal median section made through the end of the bone with a clean razor blade so as to expose a plain surface through the junction of the epiphysis and diaphysis. In every instance the left bone on each animal was used and it was sectioned through the same plain.

Both sections of the bone were rinsed in distilled water and then immediately immersed in a 5% aqueous solution of silver nitrate for one and a quarter minutes, again rinsed with distilled water and the sectioned surfaces of the bone

exposed in water to daylight until the calcified areas developed a clearly defined stain without marked discoloration of the uncalcified areas. The excess  $\text{AgNO}_3$  was then removed by immersion in a 10% sodium thiosulfate solution and after rinsing and noting the degree and extent of calcification the sections were preserved in 10% formaldehyde.

The oil tested in this assay was the same as that used in the Vitamin A assay. The oil was tested at levels of two-tenths of a milliliter and one-tenth of a milliliter daily doses, as well as being irradiated and tested straight and in dilutions of one to ten and one to one hundred.

The oil was irradiated in the following manner. Two six milliliter portions of oil were placed in a quartz test tube at a distance of 2 inches from a Cooper-Hewitt quartz Mercury Vapor Lamp made by the General Electric Vapor Lamp Company and exposure was continued for thirty minutes with stirring at five minute intervals.

This irradiated oil was fed as prepared in a one-tenth milliliter dose and in the following dilutions: 1 gm. made up to 10 ml. with cottonseed oil. The 1 to 100 dilution was made by diluting 1 cc of the 1:10 oil with 9 cc of cottonseed oil.

One group was fed no supplement and acted as a negative control while another group received one-tenth of a milliliter

of a cottonseed oil solution containing .475 International Vitamin D units. This solution was prepared from the United States Pharmacopoeia Standard Reference Oil.

The results are shown in Table XI. The plain black-eyed pea oil did not produce any healing except in two cases, each of which must be discarded either because the rat lost weight or because it did not consume enough food or both. The negative controls also failed to show any healing, as well as the irradiated oil at the one to one hundred dilution. The pea does, however, have some sterol present capable of acting as provitamin D for the irradiated oil; <sup>it</sup> produced very good results when fed straight and proved almost as efficient as the positive control when present to the extent of .1 gm. in one milliliter of solution. While exact figures mean nothing so far as the irradiated oil is concerned, it is however, evident that this oil must contain approximately 40 U.S.P.X. 1934 units per gram, which is not quite half as strong as the reference oil used.

TABLE XI

## Vitamin D Assay

Daily Dose	Rat No. & Sex	Change in Weight ing assay	Dur- ing	Degree of Heal- ing	Daily Dose	Rat No. & Sex	Change in Weight ing assay	Dur- ing	Degree of Heal- ing
		gms.					gms.		
.1 cc of Pea oil	3 M 8 M 11 M 13 M 34 F 39 F	+ 5.5 + 4 + 6 + 5 + 10.5 died		neg. neg. " " " "	positive control	9 M 10 M 25 M 40 F 32 F 37 F	+ 11 + 5 + 7 + 4 + 7 + 9.5		1+ 3+ 3+ 3+ 2+ 3+
.2 cc of Pea oil	5 M 7 M 19 M 26 M 31 ♀ 33 ♀	+ 2 + 1.5 - 1 + 4.5 - 3 + 1		neg. neg. " neg. " neg.	negative control	206 M 214 M 223 M 228 M 236 F 238 F	+ 1 + 9 + 8 + 5 + 4 + .5		neg. neg. " " " "
.1 cc Irradiat- ed Pea Oil Diluted 1: 10	12 M 17 M 20 M 27 M 30 M	+ 10 + 10.5 + 9.5 + 13 + 14		1 + 4 + 1 + 4 + 2 +	.1 cc Irradiated: Pea Oil	1 M 2 M 21 M 24 M 35 F 41 F	+ 5.5 + 8. + 9 + 7 + 6 + 11		3 + 3 + 3 + 4 + 3 + 4 +
.1 cc Irradiat- ed Pea Oil Diluted 1:100	15 M 16 M 18 M 29 M	+ 7 + 10.5 + 10.5 + 4		neg. neg. " "					

Supplementing the above data there are presented in the appendix a number of photographs of the stained bones including one for each group except that receiving .2 cc of blackeyed pea oil.

### Vitamin B<sub>1</sub> Determination

For carrying out the determination of Vitamin B<sub>1</sub> (anti-neuritic factor) the method of Sherman and Chase (1931) was used.

The diet was made as follows:

Casein (Extracted 60% ethyl alcohol)	18%
Salt Mixture (U.S.P.X. No. 1)	4%
Butterfat	8%
Codliver Oil	2%
Autoclaved Bakers Yeast	15%
Starch	53%
	<hr/>
	100%

The rats were obtained from The Sunny Hill Rabbit Farm of Washington, D. C., and weighed between 45 - 55 grams. They were put three in a cage and given the Vitamin B<sub>1</sub> free diet and distilled water ad libitum. At the end of twenty-two days preliminary period they had reached a constant weight of approximately 81.5 gms., and were then placed in separate cages and divided into five groups of six rats, each group representing the same average weight. Three of the groups were given .4 gm., .7 gm., and 1. gram respectively of ground blackeyed peas daily except Sunday (the first Sunday they received the ration). Another group received 1 gm. of ground whole wheat daily except Sunday as a positive control and the

last group received only the test diet itself. All groups were allowed the Vitamin B<sub>1</sub> free diet ad libitum. During the test each animal was weighed every three or four days. The test proper was continued for a period of sixty days at which time all the animals still alive were weighed and killed.

The results (Graph 5) proved first that the basal diet was deficient in Vitamin B<sub>1</sub>, as there was an initial increase in weight of about thirty grams over a period of twenty-two days, and control rats continued on this diet died from twenty-two to thirty-one days after reaching constant weight. The diet was comparable to that used by Sherman and Chase (1931). In view of the fact that these workers found an average increase of 20 gms. over a fourteen day period for Vitamin B<sub>1</sub> depleted rats fed 1 gm. of ground whole wheat daily except Sunday as compared with our increase of 18.7 gms.

Calculations show that the blackeyed pea has approximately twelve units of Vitamin B<sub>1</sub> per gram. However, it is unfortunate that much lower levels of blackeyed peas were not used in this test, for, in order to obtain accurate comparisons, rats receiving an average of from one to three units of the vitamin should be used.

#### Vitamin B<sub>2</sub> Determination

The method of Sherman and Bourquin(1931) was used for the determination of Vitamin B<sub>2</sub> (lactoflavin).

The diet used was as follows:

Extracted Casein	10%
Salt Mixture (U.S.P.X. No.1)	4%
Codliver Oil	2%
Butterfat	3%
Starch and Vitamin B <sub>1</sub>	<u>68%</u>
	100%

The rats used were grown in our own laboratory. They were weaned on the twenty-eighth day and placed immediately on the Vitamin B<sub>2</sub> deficient diet. After four days preliminary all rats were either losing weight or their weight was stationary so that they were immediately placed on test. The rats were placed in separate cages, divided into groups so as to be as similar as possible with regard to sex, age, litter and weight. Three groups received 0.5 gram, 1.0 gram, and 1.5 gram respectively, of the ground blackeyed peas daily, except Sunday. Another group received .2 gram of autoclaved yeast daily which was previously proved to be Vitamin B<sub>1</sub> free but which had supported growth in the absence of the Vitamin B<sub>1</sub> factor. A fifth group received only the test diet. All groups were allowed the Vitamin free diet ad libitum. The test was continued for sixty days, recording the weights of each animal every three or four days.

The results as shown in Graph Six proved, first, that the diet by itself was deficient in Vitamin B<sub>2</sub>, for the negative controls gradually lost weight. Second, that daily

increments of a material known to contain Vitamin B<sub>2</sub> could offset the deficiency and cause growth, and thirdly that the blackeyed pea has a measurable amount of Vitamin B<sub>2</sub> (lactoflavin).

Calculations show that the blackeyed pea contains from 2 to 3.2 sherman units of Vitamin B<sub>2</sub>(lactoflavin) per gram of material.



#### IV. DISCUSSION OF RESULTS

The sample of blackeyed peas used for the nitrogen distribution studies has a protein content of 22.5% (N x 6.25) and other values obtained in this laboratory and elsewhere indicate a protein range of from 21 to 26%. This is equal to and even slightly better than the values found for this constituent in most legumes, except for the soybean which approximates 38% (Bowers 1919). When the proper mixture of amino acids is present 10% of a protein in a diet is sufficient for good growth and 18% is more than enough for normal growth. In fact, any protein to be satisfactory for feeding as the sole source of protein should produce normal growth when present to the extent of 18% in the diet.

The blackeyed pea protein when present at 10% levels is totally inadequate for growth and produces only slightly better results when present at 15% and 18% levels. When the meal has been cooked, and is present at the higher level, appreciable increases in growth are noted but normal growth is not obtained. Many legumes are deficient in cystine especially when fed untreated, but the addition of this amino acid to diets containing the blackeyed pea meal, cooked or uncooked, does not entirely supplement its protein deficiencies. This addition does produce very marked

increases in growth over the unsupplemented meal and therefore the conclusion is reached that cystine is present in insufficient quantities in the pea meal.

As previously mentioned, Finks, Jones and Johns (1922) studied the Groit and Brabham variety of the cowpea and found that when the meal was cooked and supplemented with cystine normal growth ensued. This does not agree with the results found in this laboratory for the blackeyed pea, and indicates that there is an appreciable variation in the amino acid content in the different varieties of cowpea, or that different conditions of climate and soil on which the plant is cultivated greatly vary the character of its proteins. Further evidence of this line of reasoning is given by Adolph and Chiang (1935) who report a value of 1.35% for the cystine content of *Vigna Sinensis, sesquipedalis* and state that in the literature values are reported ranging from 1.25 to 6.74%. Also, in Table III values reported by the same author for the same protein obtained by different methods of extraction vary from .34 to 1.82%. Of course, some of this variation in reported values may be entirely due to different methods of extracting the protein, or to inadequate methods of determining the cystine content or both, but taking all these possibilities into consideration, it still seems true that not only the cystine content but the whole amino acid content of a protein may vary with different varieties and different methods and conditions of cultivation.

The method of cooking the blackeyed pea meal apparently has an effect on the availability of its protein content. Better results were found when the meal was cooked under ordinary pressures at the temperature of boiling water than when the meal was autoclaved. This result has been found with other protein materials but it has apparently not been studied to any great extent. Of course it is easy to postulate that the protein is denatured in some manner or that the individual amino acids are decomposed under the effects of temperature and pressure. However, in view of the extent to which autoclaving is used as a method for processing food in the canning industry it seems that much further investigation should be continued as to the cause and effect of the results noted.

It has already been shown that the blackeyed pea protein studied in this laboratory cannot promote normal growth to the conclusion that when this deficiency is supplied the material is further lacking in sufficient quantities of one or more amino acids. An indication was found that one of these acids is tryptophane but further work is necessary before definite conclusions may be made on this phase of the work.

So far the discussion has been concerned with the growth promoted by the blackeyed pea as the sole source of protein and the improvements in this growth resulting from the

addition of amino acids. While this gives an idea of the character of the deficiencies of the pea protein it does not give quite so effective a means of comparing this protein with other nitrogenous foodstuffs as is afforded by a determination of the biological value.

From a nutritive standpoint the value of a protein depends first upon its digestibility and second upon its biological value. While many writers report merely the biological value this does not seem to be sufficient for a complete interpretation of their data. For, although the biological value of a protein may be high, if its digestibility is low it is of no more value to the animal body than some other protein that shows an intermediate result for both biological value and digestibility.

The Blackeyed Pea protein has a digestibility of approximately seventy-eight percent no matter how the meal is treated before it is incorporated in the diet, and no matter what the level of protein intake. However, the cooked meal consistently shows a higher biological value than the uncooked meal. These results are interesting, first because heat treatment apparently does not alter the extent to which this protein can be broken down in the intestine and absorbed into the body, and secondly, because heat treatment does alter the protein so that the portion that is absorbed into the body is better utilized. Finks et al (1922) concluded that heat treatment changed the digestibility of the cowpea meal

but from the above results we must conclude that this is not necessarily true.

It has already been mentioned that heat treatment under pressure and increased temperature apparently alters the protein so that it is not as efficient as when atmospheric pressures and the temperature of boiling water is used. This is again shown by the lower biological value of 53% for the autoclaved meal compared with 56.5% for the milder heat treatment.

No explanation is offered for the very marked differences in these results compared with those of Niyogi, Narayana and Desai (1933). These workers reported the digestibility of 58% and a biological value of 72% for *Vigna Catjang* using the whole pea (including the seed coat). Perhaps this difference of results is caused by the difference in variety and cultivation, but if such great variations in two very similar plants can exist, then it would seem of value for someone to initiate a study of the extent to which similar or even the same variety of plants will differ under changing conditions.

As a comparison of the results found in this investigation it might be mentioned that Mitchell (1923) reports that cooked navy beans fed at 10% levels using white rats as experimental animals have a biological value of 38.4%, while Smith and Roehm (1937) reported a value of 64.3% for

well-cooked soybean meal proteins fed at 9% levels. This is in agreement with earlier growth studies that indicated the soybean proteins were superior to the cowpea proteins while the navy bean proteins were inferior.

Fifty to 60% of the blackeyed pea is carbohydrate, this is characteristic of legumes except for soybean which has a value of 27% of this constituent. At least 50% of the black-eyed pea can be hydrolyzed by enzymes to simpler sugars. This indicates, as did the preliminary growth studies, that available carbohydrates or energy producing materials are present in sufficient amount in the pea meal. Using the same method as was used in this laboratory Adolph and Kao (1934) found only 27.1% of the total carbohydrate in the whole soybean to be digested. These workers used two other "in vivo" methods of determining the biological availability of the soybean and found that the "in vitro" method was consistently lower. We conclude from this that the carbohydrate of the cowpea is probably very highly available to the animal body.

The oil content of the blackeyed pea is also similar to that of many legumes although the soybean (Bowers 1919) has the much higher value of 15%. The results show a slightly low saponification number which would indicate the presence of a relatively high quantity of unsaponifiable material. This is further indicated by the low value for soluble fatty acids. The determination of the unsaponifiable residue bears out this conclusion, and gives an explanation

for the great increase in Vitamin D potency when the oil is irradiated, for it is this portion of an oil which contains the sterols believed to be provitamin D.

The extracted oil showed no antirachitic properties whatsoever, although fed in quantities far in excess of the amount that could be obtained from the consumption of large quantities of the unextracted meal. However, the irradiated oil contained approximately forty International Units of Vitamin D per gram. This value is only half of that required by the United States Pharmacopoeia as a minimum in codliver oils. From this comparison may be seen the great improvement in Vitamin D content resulting from irradiation of the blackeyed pea oil. It is very possible that improved technique for irradiating the oil would greatly improve the Vitamin D content over the values obtained by the experiment outlined; in fact, it might even be possible to irradiate the meal itself so that when fed as a major portion in the diet it would have sufficient antirachitic properties.

Vitamin A was found to be present only in minor quantities and as Fraps (1933) has pointed out the Vitamin A potency of the stored bean decreases greatly during the period of a year. Consequently it is concluded that the blackeyed pea as such is totally inefficient so far as the fat-soluble Vitamins A and D are concerned.

Other values of the fat were not biologically assayed

but from the value reported for the unsaturated acids it seems very probable that there is no deficiency of these compounds in the pea meal.

The water soluble vitamins were studied to determine the extent to which the antineuritic factor (Vitamin B<sub>1</sub>) and lactoflavin (Vitamin B<sub>2</sub>) are present. Approximately twelve units of Vitamin B<sub>1</sub> per gram and 2 to 3.2 units of Vitamin B<sub>2</sub> per gram of blackeyed pea were found to be present in the meal. The ratio (4:1) of Vitamin B<sub>1</sub> to B<sub>2</sub> is the same in this variety of the cowpea as it is in most cereal grains and leguminous seeds. When fed in large proportions in the diet the extent to which either of these vitamins is present is probably sufficient for normal growth.

The mineral content of the pea was not studied, but definite evidence was found that it is unsatisfactory for normal growth.



## V. SUMMARY

Tables are presented from a compilation of data obtained in the literature giving values for elementary analyses, mineral content and amino acid content of *Vigna Sinensis* and related species.

A nutritive study of the *Vigna Sinensis* (Blackeyed Pea Variety) was made.

Seventy percent of the blackeyed pea nitrogen was extracted by 5% sodium chloride, 18% by .1% sodium hydroxide and 7% by ethyl alcohol (70%).

Reducing sugars are present in the meal to the extent of .12%, soluble carbohydrates to the extent of 4.7% and polysaccharides hydrolyzable by enzymes to the extent of 31%.

An analysis of the petroleum ether extracted oil is reported.

Feeding experiments indicate the blackeyed pea when fed alone will support only slight growth for periods of at least forty-five days.

Cooking the pea meal improves the character of the protein.

Cystine is a primary amino acid deficiency, tryptophane is probably the secondary amino acid deficiency.

The digestibility of the meal whether cooked or

uncooked is 78%. The biological value at 10% levels for the raw meal is 47%, for the meal heated in a water bath is 56.5%, for the autoclaved meal is 52.7%. At 5% levels the biological value of the raw meal is 67% and for the autoclaved meal is 73%.

The vitamin A content of extracted oil was found to be 2.9 international units per gram. This is equal to .4 units per gram of the blackeyed pea.

There was no Vitamin D in the extracted oil but when this was irradiated an antirachitic potency of forty international units per gram of oil was found.

Approximately twelve units of Vitamin B<sub>1</sub> (anti-neuritic factor) and two to three units of Vitamin B<sub>2</sub> (lactoflavin) were found in the blackeyed pea meal.

## VI. LITERATURE CITED

1. Acuna, E.M. (1924) The Vitamin B content of some Philippine fruits and vegetables, Philippine Agr. 12, 293-302.
2. Adolph, W. H. and H. C. Chiang (1935) The proteins of the cowpea (*Vigna Sinensis*) Chinese J. Physiol. 9, 347-54.
3. Adolph, W. H. and H. C. Hao (1934) The Biological availability of soybean carbohydrate, J. Nutrition, 7, 395.
4. Bailey, L. H. (1935) The Standard Cyclopaedia of Horticulture, 3 vols. MacMillan.
5. Bankston, F and M. L. Giddings (1934) The vitamin C content of Blackeyed peas, J. Home Econ. 26, 640-1.
6. Bhagvat, K (1935) Proteins of Indian food stuffs VI globulins of the cowpea (*Vigna Catiang Walp*) J. Indian Inst. Sci. 18A 39-47.
7. Bhagvat, K (1935) Proteins of Indian Food stuffs VIII heat coagulation of globulins from *Vigna Catiang, Walp* and *Phaseolus aconitifolius, Jacq.*, J. Indian Inst. Sci. 18A, 145-51.
8. Boas-Fixsen, M.A. (1935) A review of methods for estimating the value of proteins. Nut. Abs. and Rev. 4, 447.
9. Boas-Fixsen, M.A. and H. M. Jackson (1932) A further note on the method used to measure the nitrogenous exchange of rats, Biochem. J. 26, 1919.
10. Bowers, W.G. (1919) Some studies on the nutritive value of the soybean in the human diet. North Dakota Agr. Exp. Sta. Spec. Bull., vol. 5, P. 278.
11. Brewster and Alsberg (1919) J. Biol. Chem. 37, 367.
12. Carver, G.E. (1917) How to grow the cowpea and forty ways of preparing it as a table delicacy, Tuskegee Exp. B. 35, 1-24.
13. Cates, J.S. (1919) Is the cowpea a passing crop? Country Gentleman 84:5 No. 22nd.

## LITERATURE CITED (CONT'D.)

14. Csonka, F. A. (1935) Proteins of yeast (*Saccharomyces Cerevisial*) Jour. Biol. Chem. 109,703.
15. Finks, A.J., D.B.Jones, and C.O. Johns (1922) The role of Cystine in the dietary properties of the proteins of the cowpea, *Vigna Sinensis* and of the field pea, *Pisum Sativum*, J. Biol. Chem. 52, 403-10.
16. Forbes, E.B., R.W. Swift, A. Black and J. Kahlenberg (1935) Effects of the plane of protein intake III, J. Nutrition 10, 461.
17. Fraps, G.C. and R. Treichler (1933) Effect of storage on vitamin A in dried foods. Ind. Eng. Chem. 25, 465.
18. French, M. H. (1932) Annual report of the department of Veterinary Science for 1931, Tanganyika Territory Dept. of Vet. Sci. Pg. 32-46.
19. Goldberger, J. and G.A. Wheeler (1927) Pelleagra-preventative action of the cowpea. U. S. Public Health Service, Public Health Reports 42,2383-91.
20. Herman, V.R. (1919) Soybeans and Cowpeas for North Carolina, North Carolina Agr. Expt. B. 241, Pg. 1-40.
21. Hermans, A.J. (1930) Vitamin content of Philippine foods I, Philippine J. Sci. 41, 387-99.
22. Jansen, B.C.P. and W.F. Donath (1924) The A-vitamin content of different Indian foodstuffs and the value of the proteins of these latter to supplement the proteins of rice. Bergerl. Geneesk. Dienst. Nederland, Indie No. 1 pg. 46-98.
23. Lanham, W. B. (1937) The nutritive value of the protein of the edible portion of Haddock, Boston Mackerel and Spanish Mackerel, Master's Thesis, U. of Md.
24. Lee, C.F. and C.D. Tolle (1934) Salmon liver and salmon egg oils, Ind. Eng. Chem. 26, 446.
25. Mitchell, H.H. (1923) A method of determining the biological value of protein. J. Biol Chem. 58,873.
26. Mitchell, H.H. (1923) The biological value of proteins at different levels of intake. J. Biol. Chem. 58,905.

27. Mitchell, J. H. and W.T. Mattison (1933) Relationship between the mineral content of the soil and plants grown on the soil. South Carolina Agr. Expt. Sta. 46th Annual Report 51-2.
28. Morse, W.J. (1920) Cowpeas: culture and varieties. U. S. D.A. Farmers Bull. 1148.
29. New York State (1916) New York State Agr. Exp. Report. Pg. 116-21.
30. Niyogi, S.P., N. Narayana and B. G. Desai (1931) Studies on the nutritive value of Indian vegetable foodstuffs, Ind. J. Med. Res. 19, 859.
31. A.C.A.C., Official Methods of Analysis, 4th Ed. 1955.
32. Olmstead, W.H. (1920) Availability of carbohydrate in certain vegetables. J. Biol. Chem. 41,45.
33. Osborne, T.B. and G.F. Campbell (1897) The proteids of the cowpea (*Vigna Catjang*) J. Am. Chem. Soc. 19,494.
34. Osborne, T.B. and I.F. Harris (1903) Nitrogen in protein bodies, J. Am. Chem. Soc. 25, 323.
35. Osborne, T.B. and F.W. Heyl (1908) Hydrolysis of Vignin of the cowpea (*Vigna Sinensis*) Am. J. Physiol. 22, 362.
36. Osborne, T.B. and L. B. Mendel (1912) Beobachtungen uber Wachstum bei Futterungsversuchen mit isolierten Nahrungssubstanzen. Z. physiol. Chem. 80, 307.
37. Piper, C.V. (1913) The wild prototype of the cowpea. U.S.D.A. Bur. of Plant Indus. Circ. 124, p.29-32.
38. Prudhomme, P. (1922) Alimentary value of some Ind-Chinese leguminosae. Bull. mens. inst. nat. agr. coloniale 6, No. 50 Pg.33-41.
39. Sherman, H.C. and A. Bourquin (1931) Quantitative Determination of Vitamin G ( $B_2$ ) J.Am.Chem.Soc. 53, 3501.
40. Sherman, H.C. and E.F. Chase (1931) A quantitative study of the determination of the antineuritic Vitamin B. J. Am. Chem. Soc. 53, 3506.

## LITERATURE CITED (CONT'D.)

41. Smith, M.C. and G.H. Roehm (1937) The biological value of the proteins in Hegari and the supplemental value of certain protein concentrates used in farm animal feeding, J. Agr. Research 54, 135.
42. U. S. Pharmacopoeia-10th Decennial Revision, A 1934 revision of the text and assays for codliver oil of the Pharmacopoeia of the United States. Interim Announcement No. 2.
43. vanRossen, C. (1927) The composition of the most important vegetable foodstuffs of the Dutch East Indies. Mededeel. Algem. Proefsta. Landbouw, No. 24 Pg. 76. with summary in English C.F. Chem. Abstracts 23, 3988,29.
44. Webster, J.E. (1928) Phosphorus distribution in Grains. J. Agri. Research 37, 123.
45. Whitney, D.J. (1936) Satisfactory Blackeye beans developed Cal. Cultivator 83: 700-1 Sept. 26.

## APPENDIX

Materials Used

## Agar - Finely Ground

The Henry B. Gilpin Co., Baltimore, Md., Merck & Co.  
Rahway, N. J.

## Butterfat - In all vitamin or protein test diets.

- (1) Melted and the decanted oil filtered in a steam heated funnel through paper.
- (2) Heated with five times its weight of distilled water to about 55°C. Cooled until solid, water syphoned off. Extraction repeated until pure butterfat obtained.

## Casein - Crude powder.

Casein Manufacturing Co. of America, Inc.  
350 Madison Ve., New York, N. Y.

## Casein - Extracted free from Vitamin B and G (Sherman and Chase 1931).

400 gms. of crude casein treated with two liters 60% alcohol (by weight) stirred well for one-half hour, allowed to stand 5½ hours filtered through cheesecloth and washed with 1 liter of 60% alcohol. Again treated with 2 liters of 60% alcohol, stirred ½ hour allowed to stand 18 hours, filtered, washed, with 1 liter of 60% C<sub>2</sub>H<sub>5</sub>OH then 1 liter of 90% alcohol (by wt.) Dried at room temperature and ground.

## Casein- Vitamin free.

Guaranteed free from water soluble vitamins B, C, and G (Vitamin B complex) and fat soluble Vitamin A and D.  
The Casein Manufacturing Co. of America, Inc.

## Cod-Liver Oil - U.S.P.X. (1934 revision)

Ordinary commercial brands.

## Cod-Liver Reference Oil.

- I. U.S. Pharmacopoeia Standard Reference Oil.  
3,000 International units of Vit. A per gram.
- II. U.S. Pharmacopoeia Standard Reference Oil.  
95 International units of Vit. D per gram.

## Cottonseed Oil - Pure

Obtained from local Drug houses.

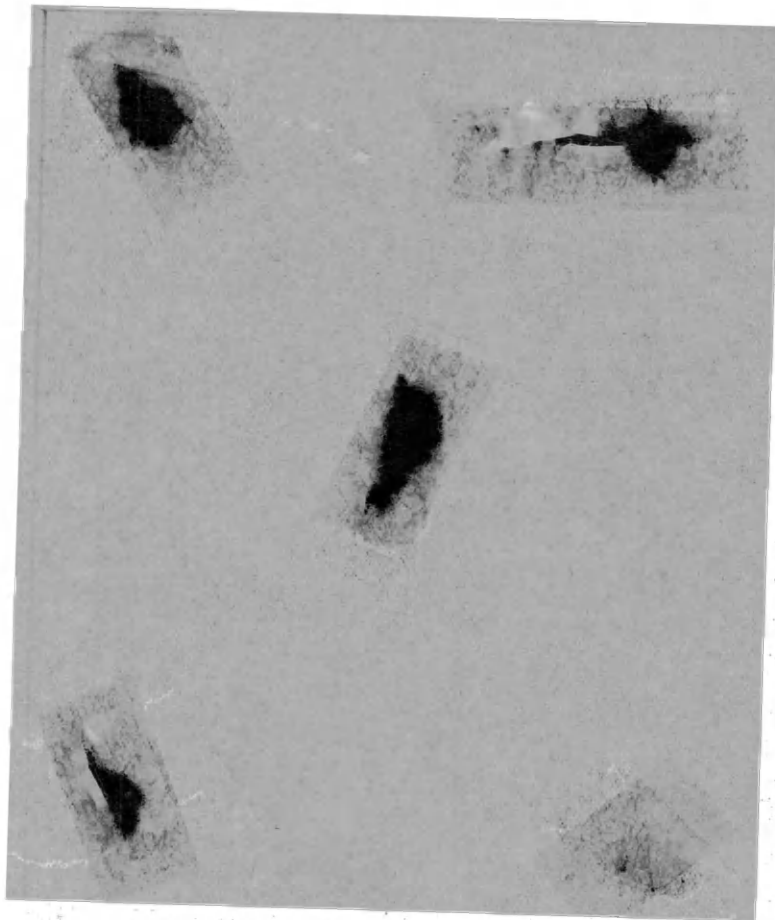
## Cystine, C.P.

The Coleman-Bell Co., Norwood, Ohio.

## APPENDIX (CONT'D.)

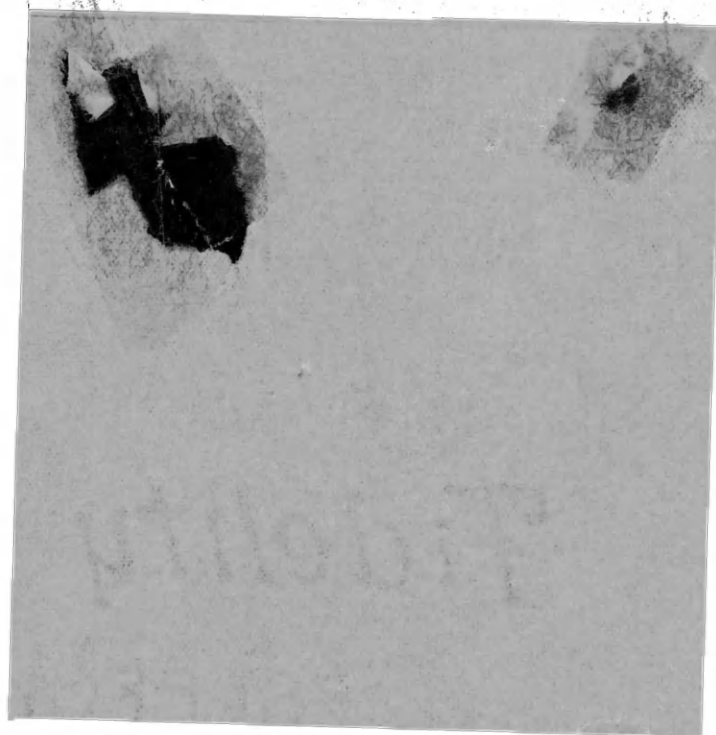
- Dextrin - Pure cornstarch mixed with small amount of water to form a firm mass which can be separated into lumps. Autoclaved for two hours at fifteen pound pressure. Dried on steam pipes and ground to a powder.
- Dextrin - Treated for Vitamin enrichment. Wheat Germs (Washburn Crosby Co., Buffalo, N. Y.) extracted with 70% ethyl alcohol by volume for 16 hours, dried on equal weight of dextrin, ground to powder.
- Labco XXX - For enriching a diet in Vitamin B and G. Water soluble Milk Vitamin Concentrate. The Borden Company, research Div. Bainbridge, N. Y.
- Starch - Commercial brands of cornstarch were used wherever starch was specified in the diet.
- Starch - With Vitamin B added (Sherman and Bourquin (1931) 1½ liters of alcohol (80% by wt.) were shaken for 1½ hours with 800 gm of freshly ground whole wheat. Filtered on a Buchner funnel and residue treated with 1000 cc of the 80% EtOH filtered again, washed with 300 cc of the alcohol. The filtrate and washings were filtered through paper, concentrated by evaporating under reduced pressure, dried at room temperature upon starch. (Extract from 50 gm. of whole wheat for every 100 gm. food mixture).
- Stock Diet - Purina Dog Chow. Ralston Purina Company, St. Louis, Missouri.  
This is a good complete foodstuff used in many nutrition laboratories as the stock diet.
- Taka-Diastase - Parke, Davis & Company, Detroit, Mich.
- Yeast - Autoclaved free from Vitamin B<sub>1</sub>. (Sherman and Bourquin 1931). Powder bakers yeast was mixed with .1 M NaOH (135 cc. for each 100 gm. of yeast) to make a smooth paste and then placed in shallow pans to a depth not to exceed one-half inch. The mixture was autoclaved 6 hours at 15" pressure. Following this the mixture was neutralized with equivalent amounts of .5N HCl. Dried at room temperature with a fan and ground to a powder.
- Yeast Foam Tablet Powder - Extremely rich in B and G. Pure Dehydrated Yeast, Northwestern Yeast Co., Chicago, Ill.
- Irradiated Yeast - Fleischmann's test pure dry Brewers yeast. Exceptionally rich in Vitamin B and G. Standard Brands, Inc. New York.





Vitamin D  
Determination

Negative Control  
Rat 28



Positive Control Rat 40  
.1cc (.5gm Standard C.L.O. in 10cc Cottonseed Oil)



Vitamin D  
Determination

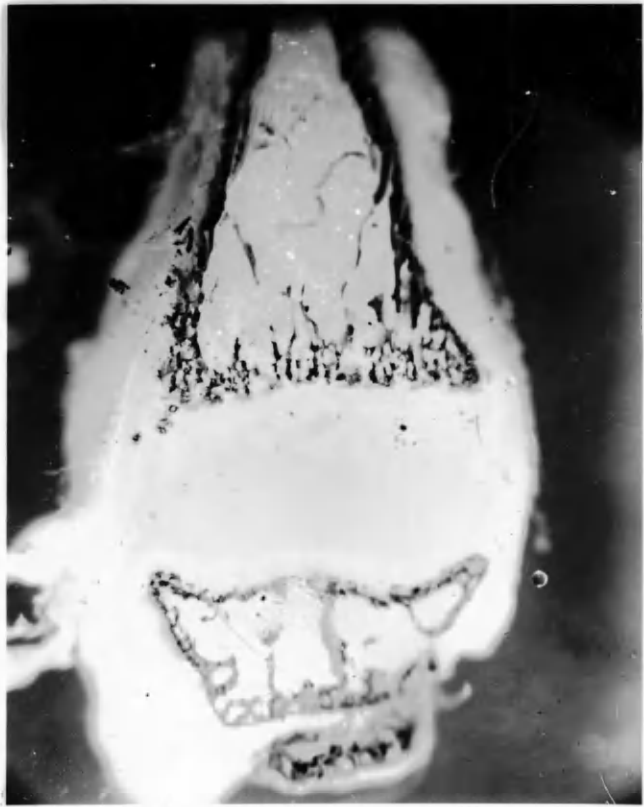
.1cc Blackeyed Pea Oil  
Rat 34



.1cc Irradiated Blackeyed Pea Oil

Rat 41

Rat 29  
Cottonseed Oil)  
-icc (1gm Irradiated Pea Oil in 100cc

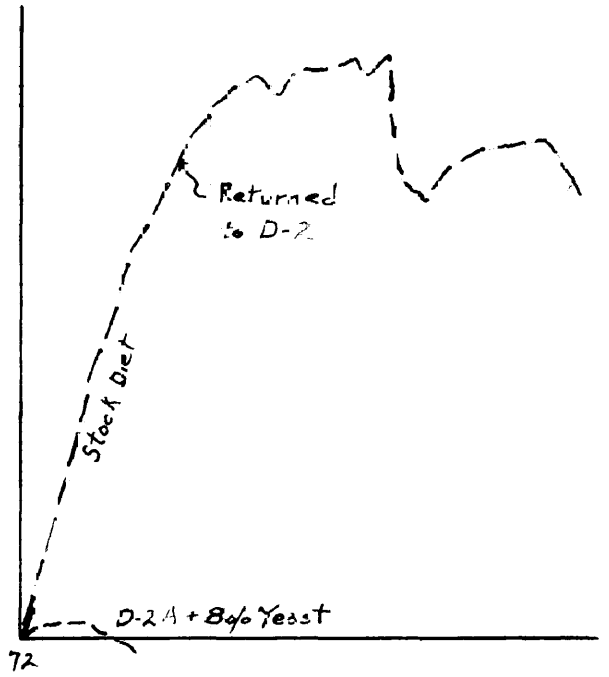
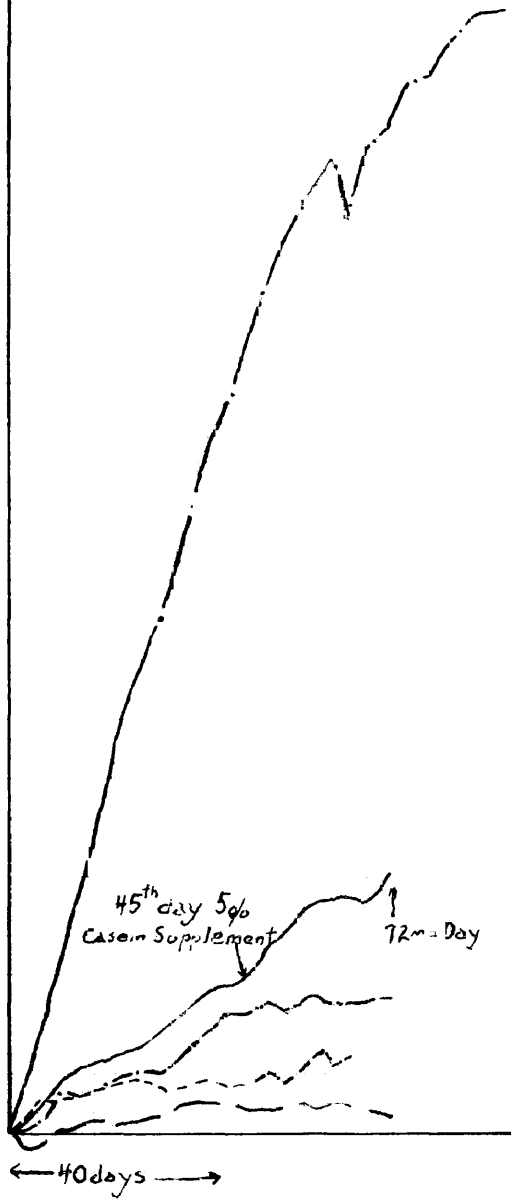
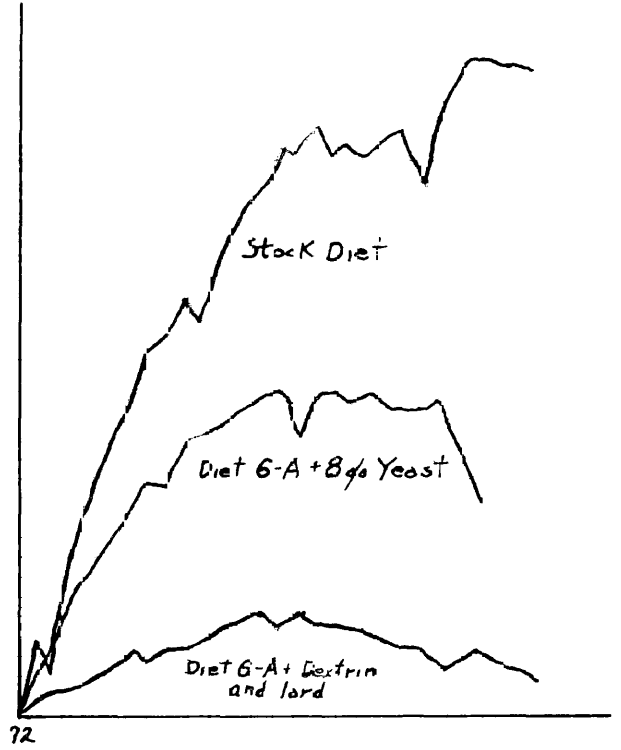
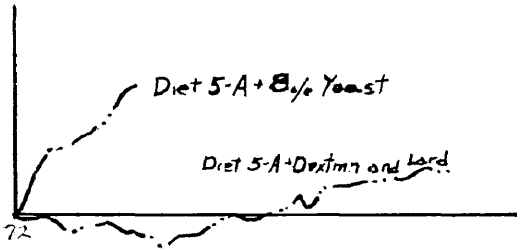


Rat 30  
Cottonseed Oil)  
-icc (1gm Irradiated O.I. in 10cc



Vitamin D  
Determination

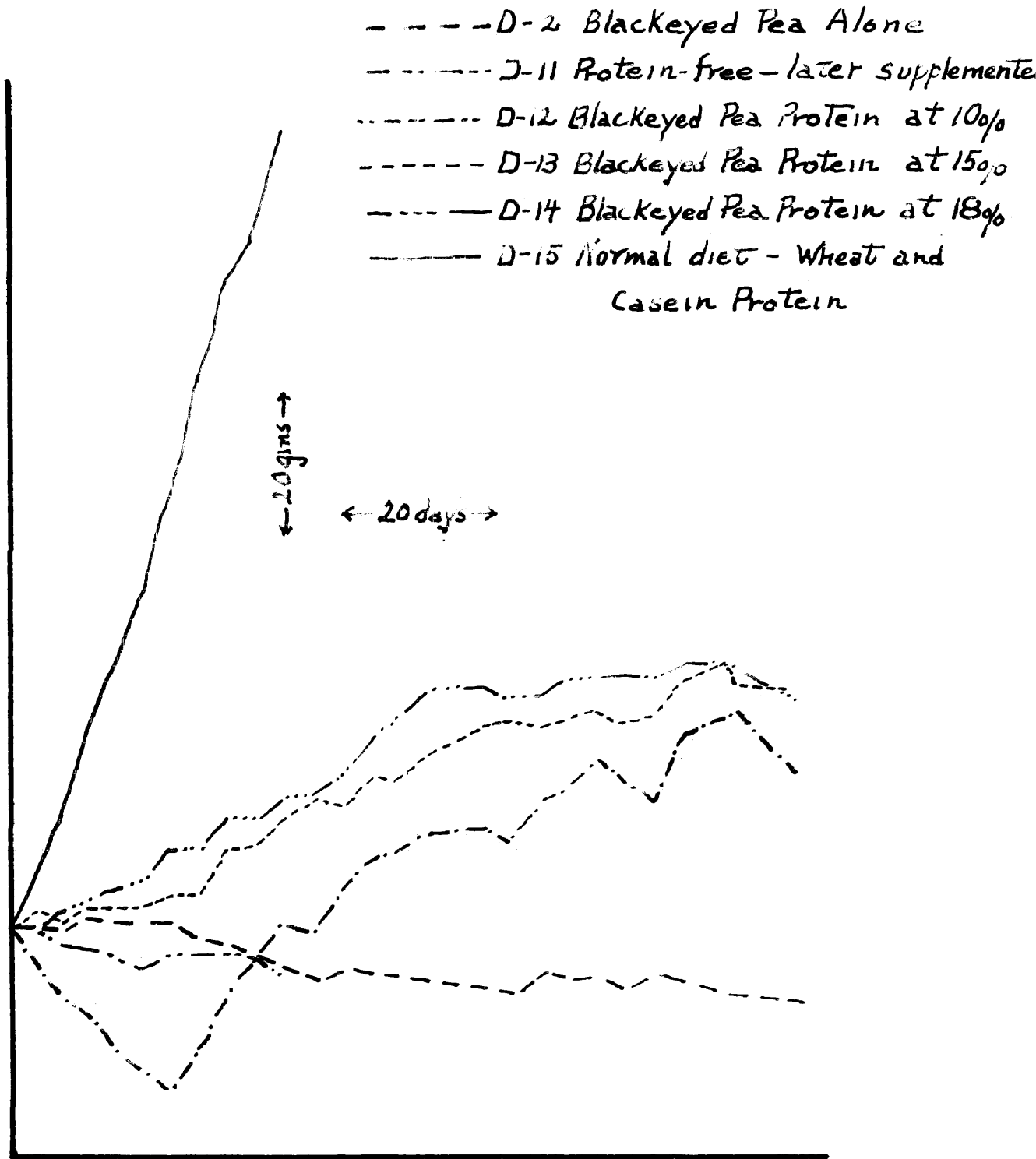
# Graph 1



- Diet 1 Stock
- - - Diet 2 Blackeyed Pea Alone
- ..... Diet 4 Blackeyed Pea 36% Salt 4%
- · - · - Diet 5 Blackeyed Pea 95% Butter 5%
- Diet 6 Blackeyed Pea 91% Salt 4% Butter 5%

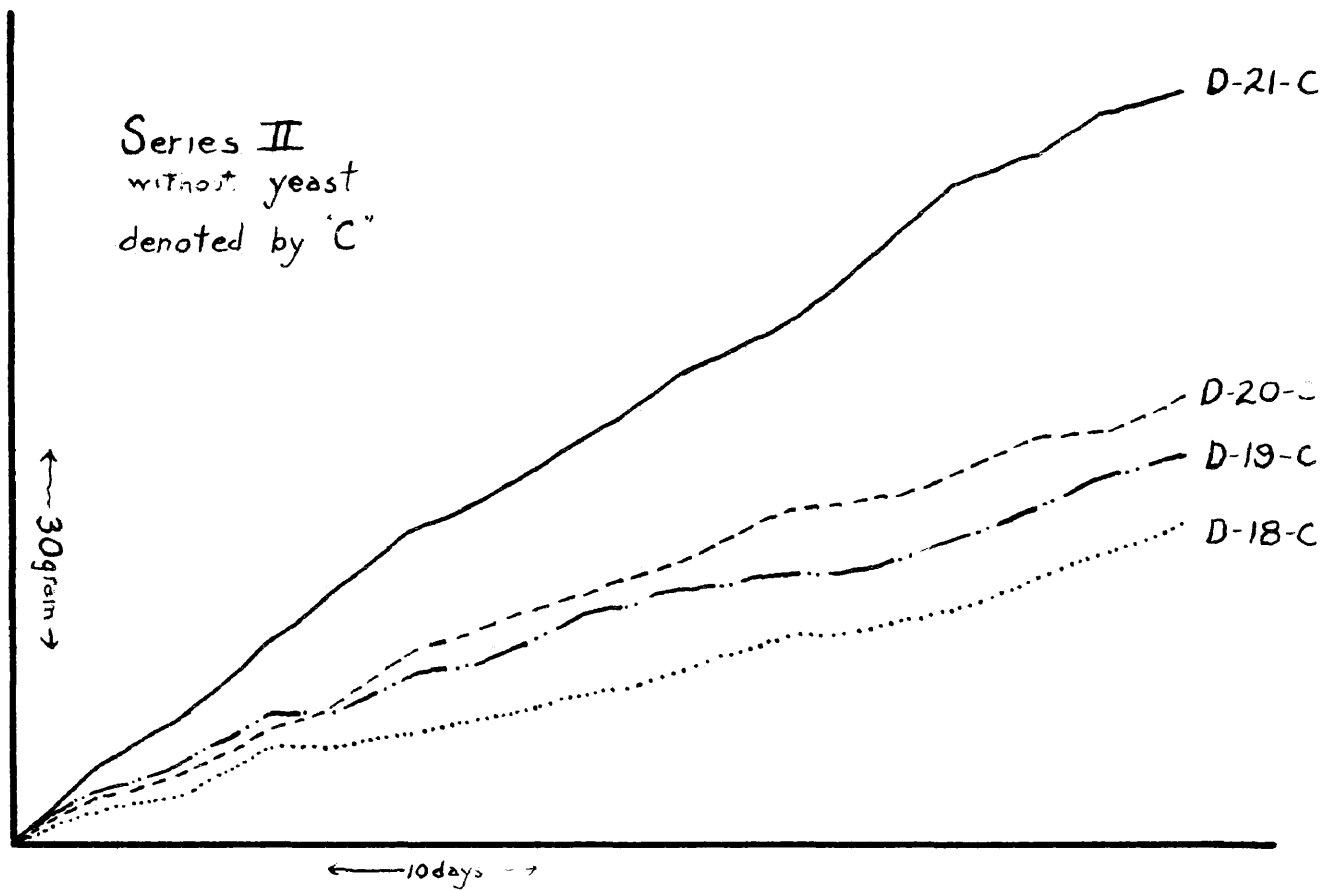
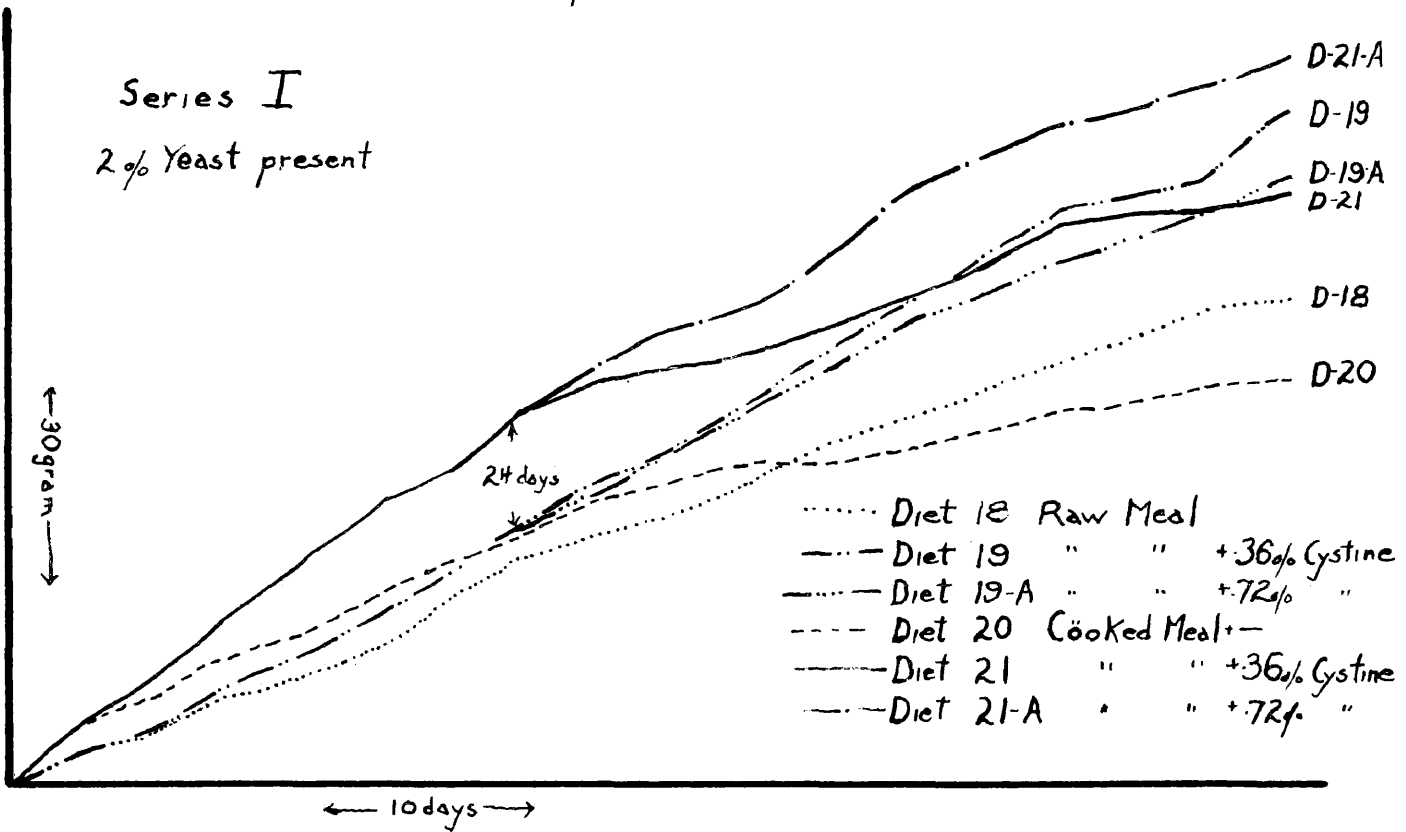
Blackeyed Pea Alone and  
Supplemented with salt  
Mixture and Butter fat

# Graph 2



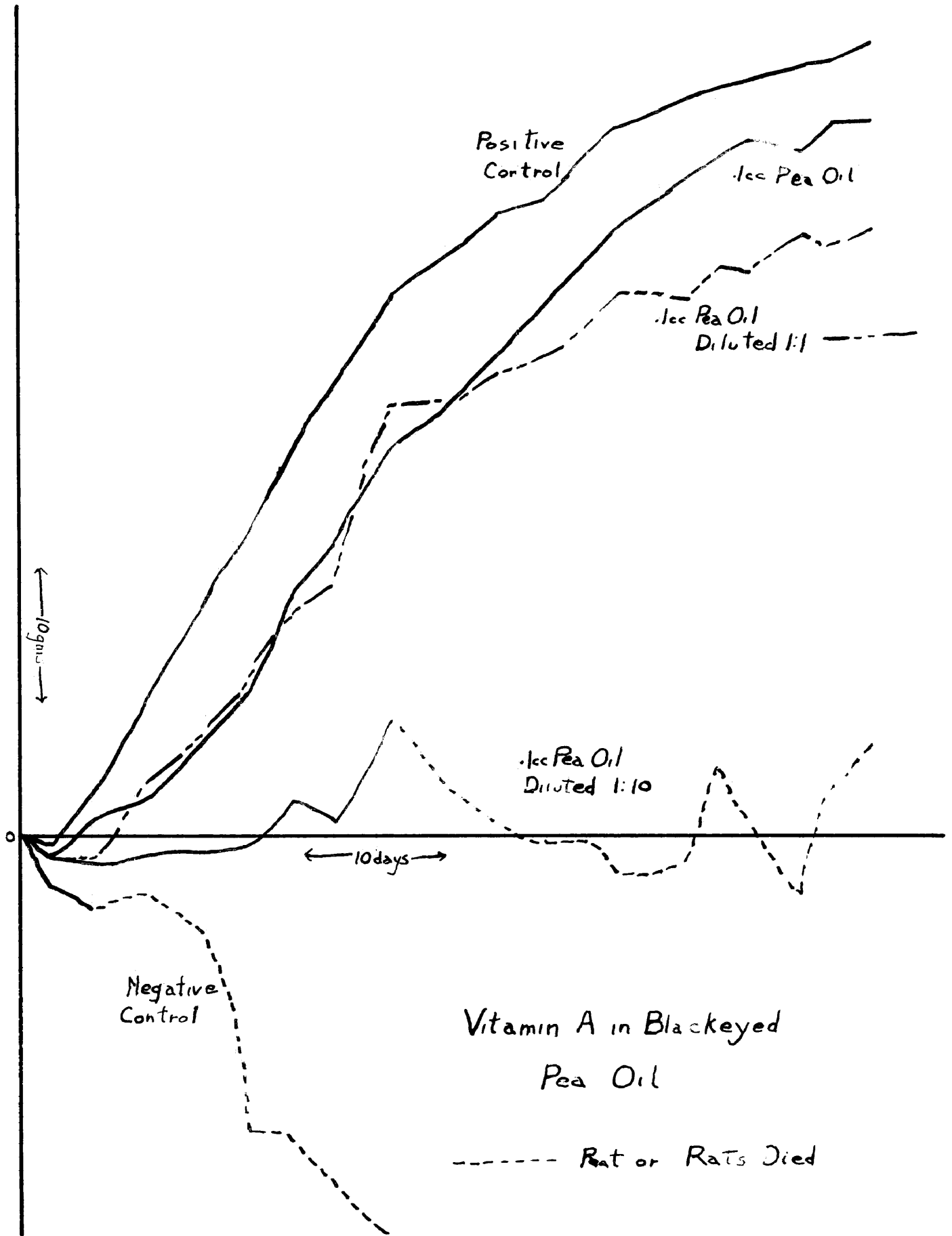
Blackeyed Pea Meal at Different Levels of Intake

# Graph 3

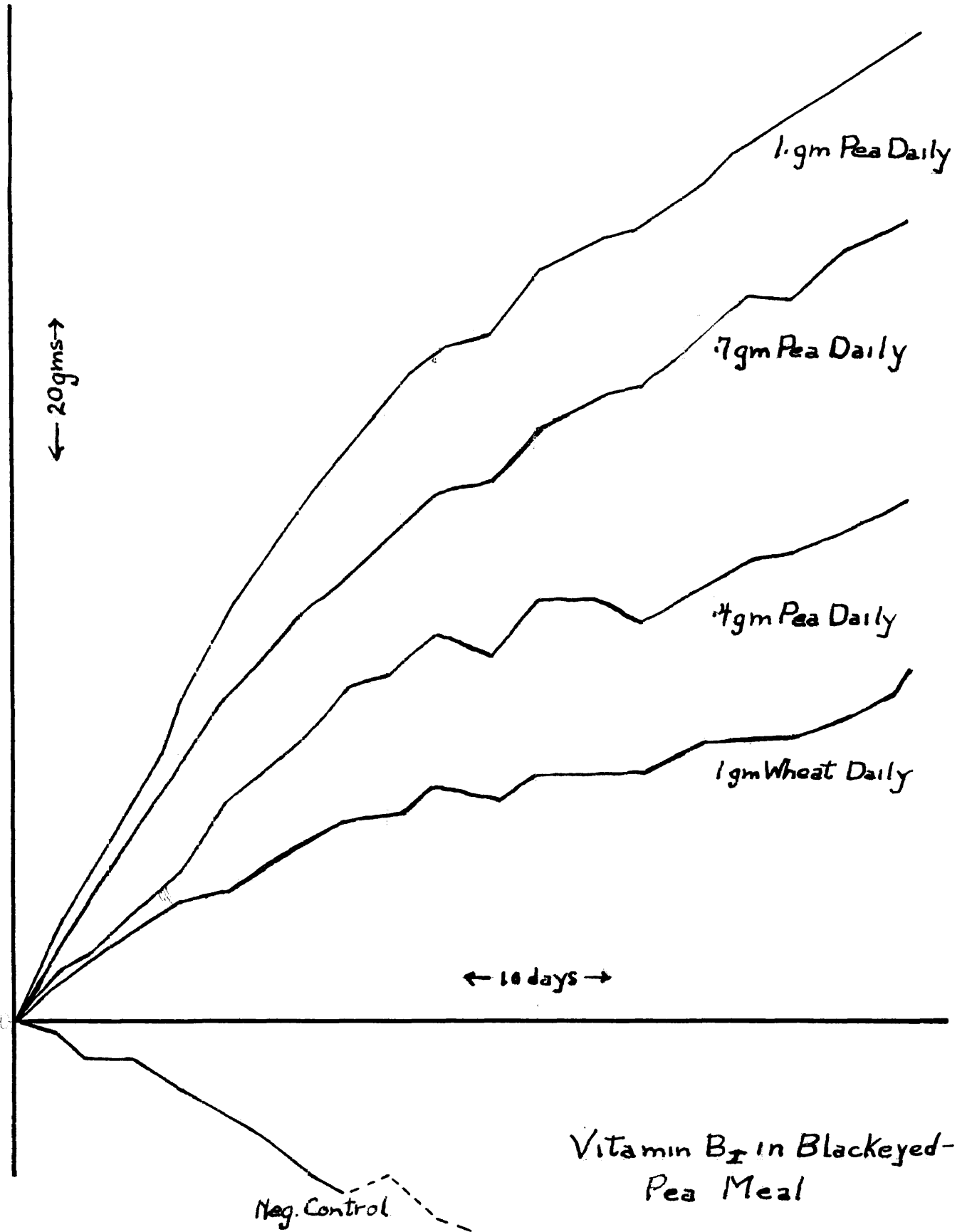


Cooked and Raw Blackeyed Pea Meal  
with and without Cystine supplements

Graph 4

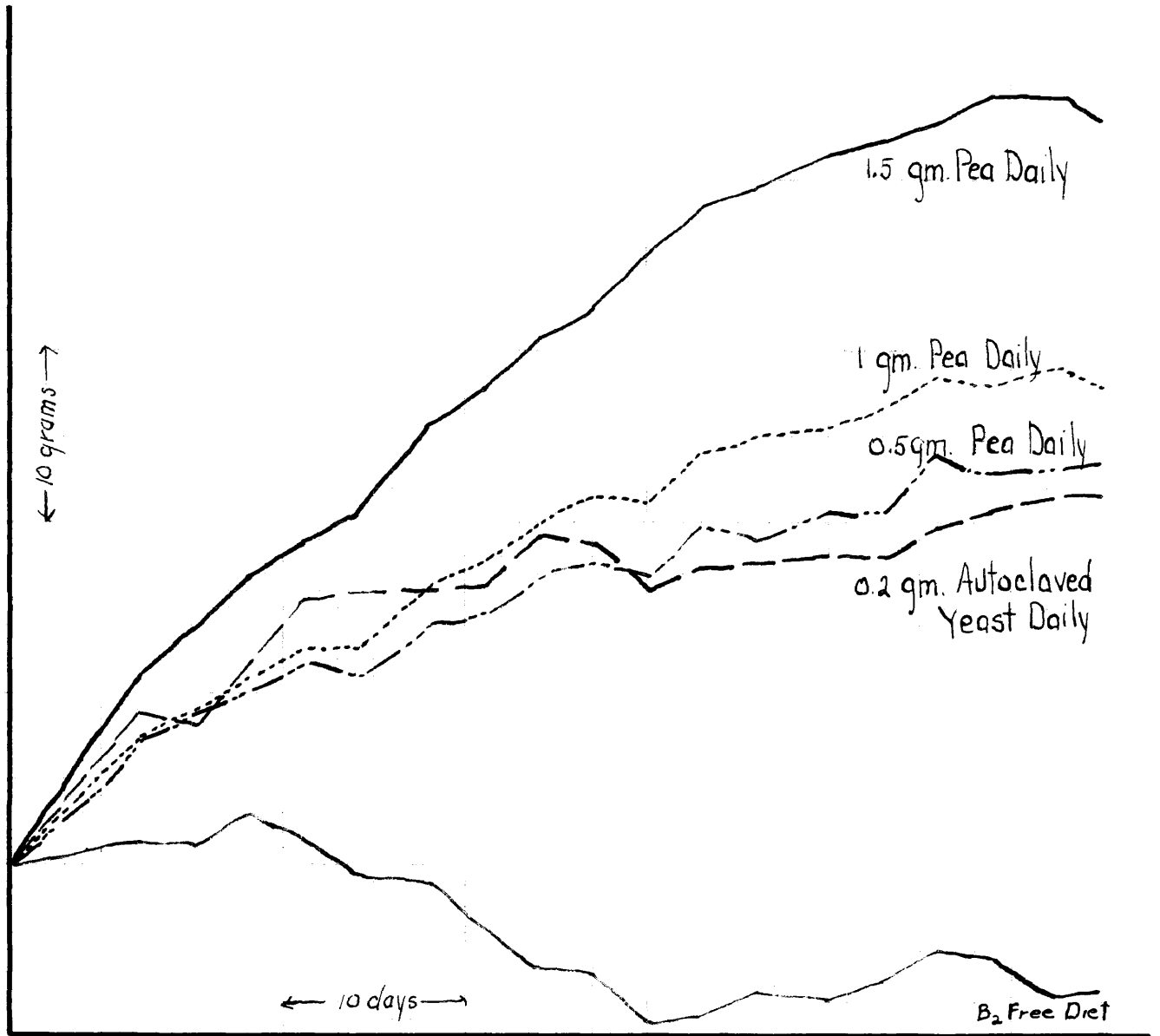


# Graph 5





Graph 6



Vitamin B<sub>2</sub> in Blackeyed Pea Meal