INDOLE DERIVATIVES IN CONNECTION WITH A DIET DEFICIENT IN TRYPTOPHANE. II.

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(Received for publication, July 8, 1929.)

The well established fact that tryptophane is necessary both for body maintenance and growth gives rise to considerable interest as to what metabolic paths this amino acid travels in the animal organism. From the various studies thus far reported, it appears that kynurenic acid is the only *definitely characterized* intermediary substance that has been related beyond doubt to the physiology of tryptophane, and kynurenic acid is generally regarded as a "shunt" product. In view of the undoubted importance of tryptophane in contrast to the rather meager knowledge available concerning the chemical alterations which it undergoes in the body, Dakin (1922, p. 97) has stated: "Further investigation of the normal metabolism of tryptophan is highly desirable." What compounds in addition to kynurenic acid play a rôle therein?

A method of approach which has appeared attractive to the writer has been that of administering possible metabolic intermediates or likely progenitors of such intermediates to animals deprived of a particular indispensable unit. Either the nitrogen balance or the change in body weight may be used as an index of the availability of a substance thus tested. Studies based upon the nitrogen balance method were undertaken by Abderhalden in 1915. The following year Asayama working in Hopkins' laboratory demonstrated that the animal's synthesis of kynurenic acid from tryptophane is not a reversible one. Rats in a condition of stress for tryptophane did not grow when given kynurenic acid. Since then, several further similar studies of tryptophane, lysine, histidine, and cystine have been published. In an earlier paper

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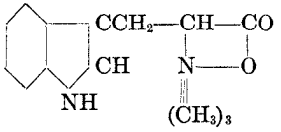
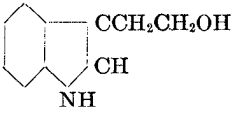
on tryptophane (1927), the writer briefly reviewed some of these investigations. Most notable were the observations of Cox and Rose (1926) and Harrow and Sherwin (1926) that *dl*-β-4-imidazole lactic acid could to a large extent replace histidine in the diet. The corresponding pyruvic acid was likewise found physiologically available, though to a lesser extent.

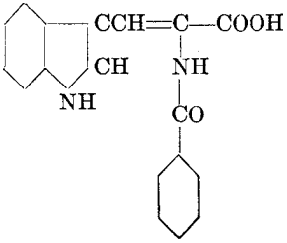
In view of these significant findings in regard to histidine, it became important to investigate in a like manner various tryptophane derivatives. 3-Indole aldehyde and *l*-β-3-indole lactic acid were found without effect when fed to rats upon a diet deficient in tryptophane (Jackson, 1927). In the present paper[1] is recorded an investigation of a series of nine additional indole derivatives with position 3 side chains as shown in the accompanying formulas.



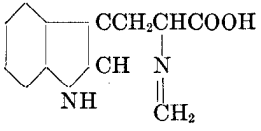
The betaine of tryptophane



Indole ethyl alcohol

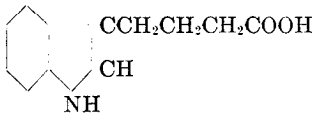


β-Indole α-benzoylamino-acrylic acid

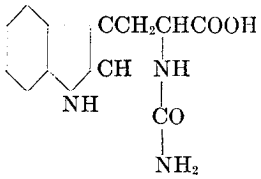


The formaldehyde-tryptophane product (Homer's formula)

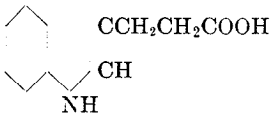
[1] A preliminary report was made at the meeting of the American Society of Biological Chemists at Ann Arbor, 1928.



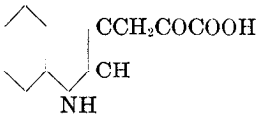
Indole butyric acid



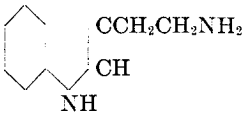
β-Indole α-uraminopropionic acid



Indole propionic acid



Indole pyruvic acid



Indole ethylamine

Of the materials enumerated, indole propionic acid, indole butyric acid, indole ethylamine, and indole ethyl alcohol may not at first appear to be particularly promising for the purpose of this study, but it must be kept in mind that all of these may at least undergo oxidation and thereby be transformed to intermediates which themselves might be suitable for tryptophane synthesis. Thus indole butyric acid by β-oxidation would be converted to indole acetic acid perhaps via indole acetaldehyde or some other equally interesting intermediate. Much the same argument holds for indole ethylamine and indole ethyl alcohol which have been shown respectively by Ewins and Laidlaw (1913) and Ward (1923) to be oxidized to indole acetic acid in the animal body. Sullivan (1922) has detected indole ethylamine in the urine of pellagrins.

Indole pyruvic acid, in view of the generally emphasized close physiological relation between α-amino acids and the corresponding pyruvic acids, appeared to be quite deserving of investigation. Moreover, Ellinger and Matsuoka (1920) have discovered that it gives rise to an increased kynurenic acid output. It possesses,

therefore, a very distinctive metabolic property in common with tryptophane. β-Indole α-benzoylaminoacrylic acid is interesting from the physiological standpoint of the dehydrogenation theory of amino acid oxidation (*cf.* Wieland and Bergel, 1924, and Dakin, 1926). Further the structural similarity of the substance to hippuric acid, which is so closely related to the corresponding amino acid glycine, is to be noted. The betaines of the amino acids found in proteins are of considerable importance—an importance accentuated by the recent discovery of ergothioneine in mammalian blood. Hypaphorine, the betaine of tryptophane, occurs in nature in the plant kingdom (*cf.* van Romburgh and Barger, 1911).

β-Indole α-uraminopropionic acid is significant since in it the amino group of tryptophane is replaced with another of common physiological importance—the urea group. Fearon and Montgomery (1924) have discussed the possible substitution of the α-amino group as a step previous to deamination (*cf.* Lippich, 1908; Dakin, 1926). The uramino derivative of tryptophane is of interest in this connection. Fearon and Montgomery themselves have in particular suggested the possibility of the chemical alteration of the $—NH_2$ to the $—N{=}CH_2$ grouping in the deamination process. The corresponding $—N{=}CH_2$ derivative of tryptophane, therefore, was also taken under consideration.

EXPERIMENTAL.

The general experimental technique described in the earlier publication was again employed with the following modifications. Owing to reports that intestinal bacteria possess the power of synthesizing tryptophane, it was thought best to confine the rats in cages equipped with false bottoms. All animals numbered 52 or beyond were thus prevented access to the feces. The lard in the diet was increased from 20 to 25 per cent, the extra 5 per cent displacing the 5 per cent of cod liver oil, which material was now given separately, 5 drops (100 mg.) per each rat per day. Vitamin B (what has hitherto been called vitamin B) was administered also separately in the form of 150 mg. of yeast concentrate[2] mixed with an equal amount of dextrin. 25 mg. of tryptophane were

[2] Lots 1030 and 1042 of Yeast Vitamine Harris were made into a mixture which was tested for use in these experiments.

added to each 100 gm. of the basal diet in order to bring about body maintenance as nearly as possible. The positive effect of any derivative might then be more accurately observed in departures of the growth curve from the horizontal line. In turn, the amount of tryptophane in the control diet was lowered from 300 to 250 mg. per 100 gm. and the derivative to be tested was incorporated in the diet in amounts equivalent to 250 mg. of tryptophane; *i.e.*, 1 or 2 equivalents, etc.

Preparation of the Indole Compounds.

The betaine of tryptophane was prepared according to van Romburgh and Barger (1911). 6.0 gm. of pure tryptophane upon methylation gave 6.5 gm. of crystalline methyl ester of the quarternary iodide of excellent quality (4.5 gm. of the nitrate were recovered from the mother liquor) which was hydrolyzed and converted to the nitrate. This in turn was treated with sodium carbonate solution to give the crude free base (yield 4.4 gm.). Recrystallization from 95 per cent alcohol and ether gave 3.7 gm. of beautiful white crystals of the dihydrate. The following data are for the anhydrous substance. M. p., 249° (corrected); m. p. (van Romburgh and Barger), about 255°. Total nitrogen,[3] per cent: found, 11.05; theoretical, 11.38.

The β-indole α-benzoylaminoacrylic acid was prepared according to Ellinger and Flamand (1908). From 3.0 gm of 3-indole aldehyde were secured about 4 gm. of the crude product which upon four fractional crystallizations from 70 per cent alcohol with simultaneous bone-black treatments yielded 1.7 gm. of white crystals. M. p., 232–234° (corrected); m. p. (Ellinger and Flamand), 232–234°. Total nitrogen, per cent: found, 9.01; theoretical, 9.15.

The indole butyric acid[4] was synthesized by applying the Fischer indole synthesis to the phenylhydrazone of ethyl hydrogen α-ketopimelate. The acid crystallized from a benzene-petroleum ether mixture as glistening rhombic plates. M. p., 123–124° (corrected). Total nitrogen, per cent: found, 6.77; theoretical, 6.90.

The indole propionic acid[5] was prepared according to Kalb, Schweizer, and Schimpf (1926). The material after a final crystallization from hot water consisted of white needles. M. p., 133–134° (corrected); m. p. (Kalb, Schweizer, and Schimpf), 134°. Total nitrogen, per cent: found, 7.32; theoretical, 7.41.

The indole ethylamine was prepared according to Majima and Hoshino (1925). From 15.6 gm. of indole were secured 13 gm. of indole acetonitrile which upon reduction yielded 7 gm. of the crude base. This was finally

[3] All nitrogen determinations were made by the Kjeldahl method.

[4] A complete acount of the synthesis will be published shortly in collaboration with R. H. F. Manske.

[5] This material was kindly supplied by Dr. Manske.

purified by adding an ether solution of hydrochloric acid to the material dissolved in a mixture of 400 cc. of ether and 40 cc. methyl alcohol. The material was once more crystallized from alcohol and ether. The final yield of the hydrochloride was 3.6 gm. The crystals were white with just a very slight tinge of lemon-yellow. M. p., 251–253° (corrected); m.p. (Majima and Hoshino), 248–249°. Total nitrogen, per cent: found, 14.04; theoretical, 14.25.

The indole ethyl alcohol was prepared according to the elegant biological method of Felix Ehrlich (1912). 10 gm. of tryptophane gave 7.2 gm. of nicely crystalline crude tryptophol.[6] (The theoretical yield here would be 7.9 gm.) This was recrystallized from 25 per cent alcohol to give 3.7 gm. of first crystals and 2.6 gm. of additional very slightly colored material. The first fraction consisted of beautiful colorless plates. M. p., 57–59° (corrected); m. p. (Ehrlich), 59°. Total nitrogen, per cent: found, 8.62; theoretical, 8.69.

The formaldehyde condensation product of tryptophane was prepared according to Homer (1913). 2.0 gm. of pure tryptophane yielded 2.2 gm. of the derivative. The substance was slightly yellow, as described by Homer. M. p., 230–240° (corrected); m. p. (Homer), 225–240° and 235–240°. Total nitrogen, per cent: found, 10.75; theoretical, 11.11. It seems somewhat doubtful that this substance contains the —N=CH_2 group as suggested by Homer. Her work shows that the second molecule of water of crystallization apparently cannot be removed under the temperature which brings about decomposition of the compound. Furthermore, Franzen and Fellmer (1917) have shown that this extra molecule of "water of crystallization" is characteristic of the formaldehyde condensation products of other amino acids and also of various salts of these products. They, therefore, have suggested the —NH—CH_2—OH group instead of —N=CH_2.

The β-indole α-uraminopropionic acid was not found described in the literature. It was prepared in general according to the directions of Lippich (1908) for the production of corresponding derivatives from other amino acids. 5 gm. of pure tryptophane were refluxed for 5 hours with 12.8 gm. of urea and 320 cc. of saturated barium hydroxide. The barium was removed with carbon dioxide and the filtrate was evaporated *in vacuo* to a volume of 75 cc. Acidification with acetic acid gave 4.7 gm. of crude material. Three crystallizations out of 50 per cent alcohol gave 3.30 gm. of crystalline substance (prisms and plates). M. p., 200° (corrected). Total nitrogen, per cent: found, 16.80; theoretical, 17.00. It may be noted that this material was pure white until the second crystallization. It then assumed a very slight purplish cast both in the crystals and in the mother liquor; the color still remained after the final crystallization. This phenomenon is suggestive of the pinkish cast which even pure 3-indole acetic acid may assume. The identity of the uramino derivative of tryptophane

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[6] The writer wishes to thank Dr. Frey of the Fleischmann Laboratories, New York, for a generous supply of starch-free yeast.

was further established by converting a small amount of it to the hydantoin which has previously been described by Majima and Kotake (1922).

The indole pyruvic acid was prepared according to Gränacher, Gerö, and Schelling (1924). 3-Indole aldehyde was condensd with rhodanine. The resulting substituted rhodanine was hydrolyzed to indole sulfhydryl-acrylic acid from which the sulfur was eliminated to give the desired indole pyruvic acid. Preparation 1: The crude pyruvic acid prepared from 3 gm. of the indole aldehyde was twice precipitated from boiling acetic acid to give 0.6 gm. of brown micro crystals (containing 1 mol of acetic acid of crystallization). Preparation 2: The crude pyruvic acid prepared from 5 gm. of the indole aldehyde was twice fractionally precipitated from ether with petroleum ether to give 1.8 gm. of a very light brown material. Total nitrogen, per cent: found, 7.27; theoretical, 6.90. Examination of Preparation 2 for amino nitrogen in the Van Syke apparatus showed that the "amino nitrogen" was 3 per cent of the total nitrogen. The melting points of both preparations described here were indefinite; it seems that a *fairly sharp* melting point for indole pyruvic acid has not been reported.

The tryptophane used both in the construction of diets and in the synthesis of the derivatives was prepared in quantity essentially according to Onslow's (1921) modification of the original method of Hopkins and Cole (1901). The preparation was beautifully crystalline. Total nitrogen, per cent: found, 13.60; theoretical, 13.72. A polarimetric examination revealed it to be the levo form.

Diets.

The diets employed are indicated in Table I. Generally the rats were placed upon Diet 210 until they reached 115 to 120 gm. in body weight, and then were transferred to the tryptophane-deficient diet, No. 211, for a period of about 44 days. Most of the animals eventually exhibited a remarkably constant body weight maintenance. Next, the derivative to be studied along with sufficient sodium bicarbonate to neutralize any free acid present was introduced in 1 or 2 equivalents (equivalents of 250 mg. of tryptophane per 100 gm. of food; see Table I) most often for 24 days. The deficient diet was then restored for a period, and finally tryptophane was incorporated in the diet to demonstrate that the animal still possessed capacity for growth. Thus each experiment was controlled within itself. In addition, separate animals were always kept on both the deficient diet and the deficient diet plus tryptophane to show simultaneously that no abnormal environmental factors were operating.

TABLE I.

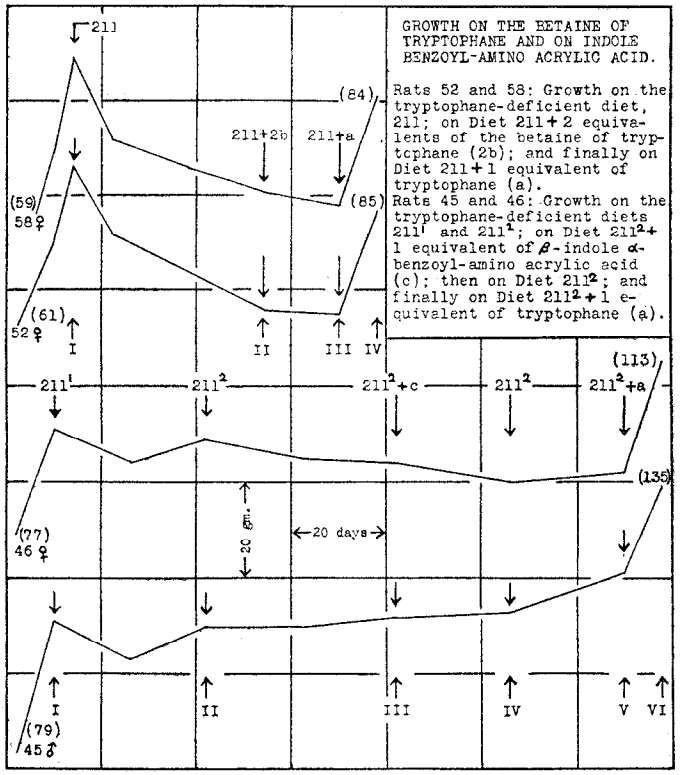
Composition of Diets.

	Diet No.			Diet 211 supplemented with derivatives.		
	210	211	E	Diet No.	Compound.	Amount of compound added per 100 gm. Diet 211.
	gm.	*gm.*	*gm.*			*mg.*
Casein, technical.	18.0			211 + a†	Tryptophane.	250
Casein digest (digested				211 + b	Betaine of tryptophane.	302
in acid).		14.4		211 + c	β-Indole α-benzoylaminoacrylic acid.	375
Cystine.		0.3		211 + d	Indole butyric acid.	249
Tyrosine.		0.3		211 + e	Indole propionic acid.	232
Tryptophane.		0.025		211 + f	Indole ethylamine hydrochloride.	241
Dextrin, white.	36.0	39.0	50.0	211 + g	Indole ethyl alcohol.	197
Sucrose.	15.0	15.0	25.0	211 + h	Formaldehyde-tryptophane product.	309
Salt mixture.*	4.0	4.0		211 + i	β-Indole α-uraminopropionic acid.	303
Agar (ground).	2.0	2.0		211 + j	Indole pyruvic acid.	249
Lard.	25.0	25.0	25.0			(323)‡
Total.....	100.0	100.0	100.0			

* The salt mixture used was prepared according to Osborne and Mendel (1919).

† Diets containing the indole derivatives are designated by appending letters to 211. The single letter stands for one equivalent of the derivative; when other than one equivalent is employed, the proper coefficient is inserted.

‡ In the case of Preparation 1 (j), 323 mg. were used in order to allow for the 1 molecule of acetic acid of crystallization.



CHARTS I to VII. The initial portions of the growth curves represent growth upon the unhydrolyzed-casein diet, Diet 210 (no legend shown). The downward arrows indicate dietary changes. The first number above a downward arrow denotes the diet introduced. Upward arrows indicate points and intervals for the purpose of describing the sequence of the experiments and for the purpose of recording food consumption (Tables II and III). In Tables II and III, Period I is employed always to designate 12 days on Diet 210, ending at the point marked I in the charts. Either three or four animals were used in connection with the testing of any one derivative, although the growth curves for only one or two are presented.

CHART I. Diet 211, which was fed in an experiment upon a group of animals including Rats 52 and 58, was prepared from a casein digest to which the cystine, tyrosine, and tryptophane were added in the wet way. There were indications that the subsequent drying down process caused the tryptophane to decompose. Diets 211^1 and 211^2 differ from Diet 211 only in containing 40 and 30 mg. of added tryptophane, respectively, instead of 25 mg. 2 equivalents of the indole benzoylaminoacrylic acid were not found to give different results than are shown in this chart for 1 equivalent.

DISCUSSION.

It is clear from Charts I to IV that with the exception of the indole pyruvic acid the derivatives employed had no appreciable influence upon the growth curves. Notwithstanding the close

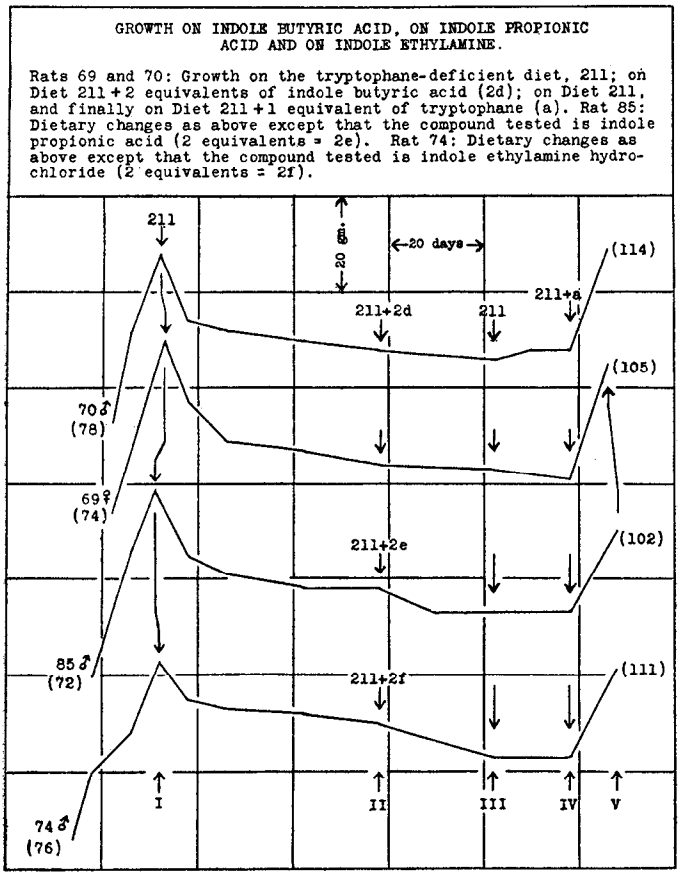


CHART II.

structural relations of the various compounds to tryptophane and the rather superficial chemical alterations necessary to convert them to that product, the organism of the rat did not utilize them for growth. Further brief experiments with indole acetic acid,

indole acetonitrile, and isatin[7] yielded wholly negative results. These results combined with those cited earlier in the paper serve

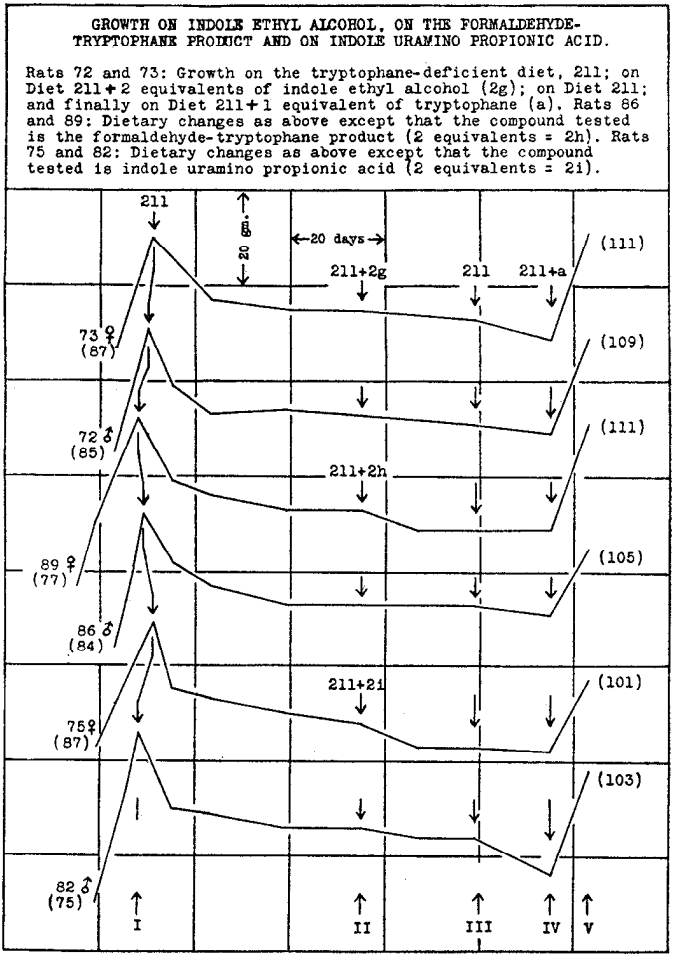


CHART III.

to show how very specific the animal is in its requirements not only for the indole ring—and for an indole ring substituted at

[7] The finding of Whipple and Smith (1928) that tryptophane and isatin both give rise to increased bile salt prompted this experiment.

position 3—but in addition for a side chain of just a certain structure. This exacting type of demand stands in close agreement with that observed in the cases of lysine, histidine, and cystine. For example, the corresponding propionic acid derivative does not

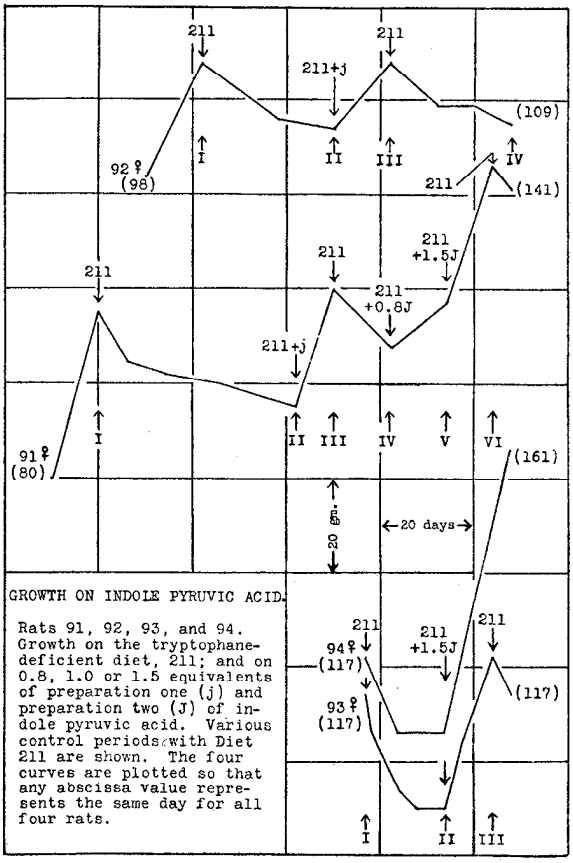


CHART IV.

replace tryptophane, histidine, or cystine in the diet. Apparently few substitutes are acceptable. The failure of indole ethylamine to replace tryptophane stands in agreement in a measure with the finding of Ewins and Laidlaw (1913) that indole ethylamine does not give rise to kynurenic acid; likewise the failure of the

betaine of tryptophane, with the observation of Eagles and Cox (1928) that ergothioneine, the betaine of thiolhistidine, is not appreciably converted to histidine; and finally the failure of the β-indole α-uraminopropionic acid, with the fact that the uramino derivatives of several α-amino acids are excreted unchanged. In general, there is considerable evidence that conjugation of the amino groups of amino acids with alkyl and acyl radicals results in a type of derivative which is quite resistant to chemical alteration in the animal body.

In sharp contrast to the results just discussed stand those secured in experiments with indole pyruvic acid. Chart IV shows the prompt, rapid, and unmistakable body weight increments resulting from the administration of this derivative. Besides the many demonstrations in the literature of the close physiological relations between amino acids and the corresponding pyruvic acids, one recalls here the finding of Harrow and Sherwin (1926) that imidazole pyruvic acid will to a certain extent replace histidine in the diet. The fact that indole pyruvic acid can thus serve in lieu of tryptophane has a bearing on the kynurenic acid formation from indole pyruvic acid in that it emphasizes the possibility already pointed out of the indole pyruvic acid passing through the tryptophane stage in its conversion to the kynurenic acid. No claim can be made, of course, that indole pyruvic acid is necessarily a normal intermediate in tryptophane metabolism. However, this relation may exist, and, in any case, the two substances are certainly physiologically closely connected. Heft and Sherwin[8] have very briefly reported the failure of indole pyruvic acid to effect growth in the absence of tryptophane. The writer offers no explanation of the difference between Heft and Sherwin's finding and his own. It must be kept in mind, of course, that indole pyruvic acid is difficult to prepare in quantity and in a high state of purity.

Criticism of the Method Used.

Sensitiveness.—Chart V shows the growth curves for two sets of rats which were brought into approximate weight maintenance

[8] On p. 155 of Hawk, P. B., and Bergeim, O., Practical physiological chemistry (Philadelphia, 9th edition (1926)), there occurs the sentence: "Heft and Sherwin have found that neither indolepyruvic acid,indolelactic acid nor kynurenic acid can replace tryptophane in the diet."

and then were given in one case 50 mg. (0.2 equivalent) and in the other, 250 mg. (1 equivalent) of tryptophane per 100 gm. of food. There is exhibited a definite growth response to the 50 mg. addi-

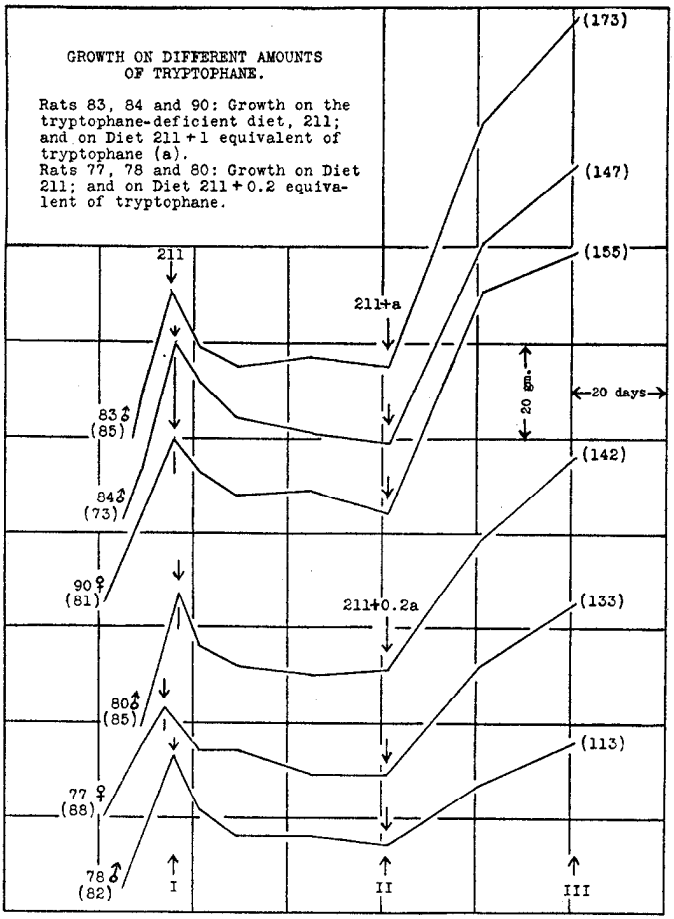


CHART V.

tion and, of course, a still greater and more nearly optimal response to the 250 mg. addition. A larger number of animals fed finely graded dosages, say, between 10 and 100 mg. should give,

when averaged, a fan-shaped set of curves which could be used as the basis for a method of assay of a protein for tryptophane much as Sherman and Woods (1925) have employed for cystine and have suggested for other amino acids. It should be especially pointed out here that of those derivatives fed in 2 equivalent amounts, 10

TABLE II.

Average Daily Food Consumption in Gm.

Rat No.	Period.					
	I	I–II	II–III	III–IV	IV–V	V–VI
58	8.1	3.4	2.8	4.9		
52	7.2	3.4	2.9	5.0		
46	7.6	5.1	4.2	4.0	4.5	7.0
45	8.4	4.5	4.6	4.4	5.0	5.1
70	8.9	4.4	4.1	4.4	7.0	
69	7.5	3.8	3.6	3.7	6.1	
85	7.5	4.5	3.8	4.5	5.5	
74	6.9	5.0	4.2	4.6	5.4	
73	8.1	5.2	4.2	4.2	7.0	
72	8.2	4.2	4.1	4.1	6.0	
89	7.6	4.5	4.3	4.7	6.6	
86	6.8	4.5	4.1	4.4	6.0	
75	6.2	4.8	4.2	4.0	5.1	
82	8.0	4.4	3.6	3.6	7.0	
92	6.4	5.7	5.9	4.6		
91	7.6	5.2	7.3	5.1	5.1	7.3
93		4.1	6.7			
94		5.4	10.1			
83	9.1	5.0	8.1			
84	7.5	5.0	6.7			
90	7.5	4.8	7.5			
80	7.8	4.5	6.5			
77	6.5	5.5	7.3			
78	8.3	4.8	6.1			
44	7.7	5.6	4.6	6.2		

times as many molecules were administered as were given to the rats receiving 50 mg. of tryptophane per 100 gm. of basal diet. In other words, if only 10 per cent of a derivative were converted to tryptophane or to some essential intermediate, the effect would still be easily recognizable. Undoubtedly even a smaller conversion could be detected.

Food Consumption.—Food consumptions were always carefully measured and recorded. The values for these are given in Tables II and III. They show the same general principle that has been emphasized before: Food consumption increases when tryptophane is included in the tryptophane-deficient diet and decreases when this amino acid is omitted. Mitchell (1927) has vigorously attacked all experiments of the type described in this paper on the grounds that a rat may well eat more of a deficient diet after addition of even just a few mg. of the material previously missing, though the supplement is homogeneously dispersed through 100 gm. of the basal diet—because the added material may alter the "flavor, odor, and texture" of the diet better to suit the taste and to whet the appetite of the rat. It is quite difficult to believe

TABLE III.

Average Daily Food Consumption in Gm.

Rat No.	Period.				
	I–III	II–III	III–IV	III–V	V–VI
76	4.1	4.1	4.1 + 2.0		
68	4.1	4.1		4.1	4.1 + 2.0
67	4.6	4.3	4.3 + 2.0		
71	4.6	4.3		4.3	4.3 + 2.0
81	4.0	3.6	3.6 + 2.0		
79	4.0	3.6		3.6	3.6 + 2.0

that such a small amount of one amino acid could exert such an enormous condimental effect when the diet in general already contains some of this amino acid itself and in addition 15 or 20 per cent of other amino acids in the free state—some bitter and some sweet. It is still more difficult to believe that such a principle holds for each of the several different amino acids so far studied, all of which behave similarily in this matter of food consumption. Rose (1928) in replying to Mitchell has adduced logical evidence that an animal under the conditions in question eats more because of a stimulation of general metabolism, which in turn results in appetite increment. The growth is not the result of a condiment, but is a register of increased chemical activity in the cells brought about by the supplying of an indispensable constituent heretofore available in amounts too small for optimum cellular activity.

It was decided to study this question from two angles: (1) Does the rat eat more because of a condimental effect? (2) Is it possible to have test and control animals ingest the same amount of food,

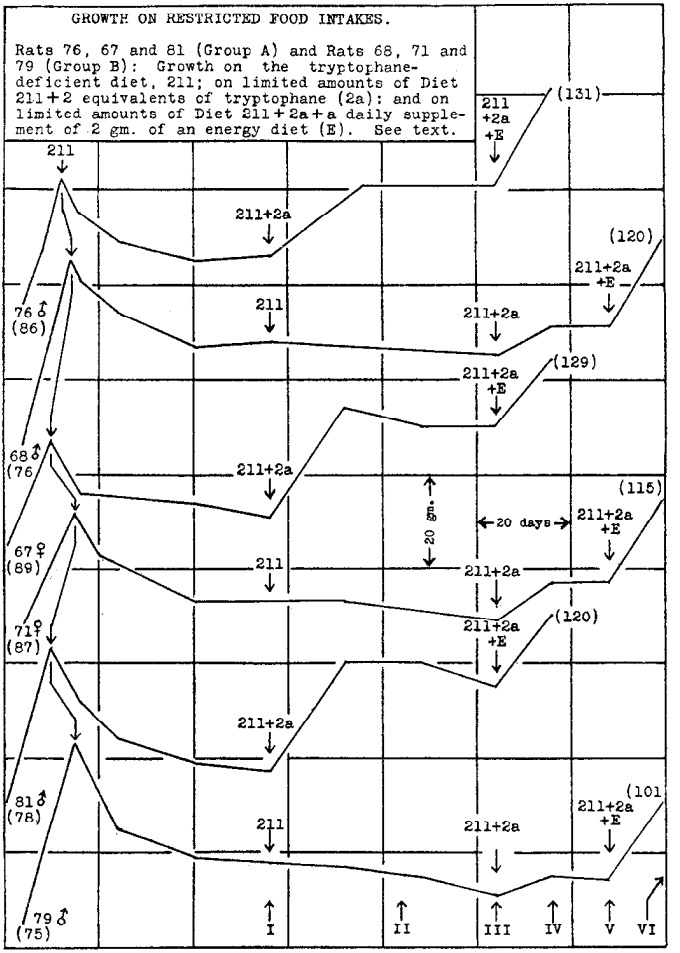


CHART VI.

yet exhibit a difference in growth rates as distinctive and characteristic as is secured with the method now in use? To investigate the first question animals brought into approximate weight

maintenance upon the tryptophane-deficient diet were given daily 25 mg. of tryptophane in the vitamin B mixture apart from the basal diet. Fair growth ensued. The data are not presented here inasmuch as Berg and Rose (1929) have recently demonstrated the same thing in another connection. That the tryptophane given in this manner, *i.e. separately*, and mixed with a small constant amount of yeast concentrate, which the animal eats at once, should affect the "flavor, odor, and texture" of the basal diet which the animal eats *ad libitum* over the whole 24 hours, is beyond credulity.

The second question was studied by bringing a number of rats into approximate weight maintenance on the deficient diet. Six were chosen in three pairs, each pair being of the same sex and exhibiting no greater than a gm. difference in weight (see Chart VI). Three of these (Rats 68, 71, 79, Group B), one from each pair, were continued upon the deficient diet. The other three (Rats 76, 67, 81, Group A), were then restricted to the food consumption of their respective mates, however, with tryptophane added at a 500 mg. level so that it could not possibly be a limiting factor. One-fourth of what one of the pair ate in 4 days was given daily to the second of the pair during the next 4 days (Period I-III). It was estimated that food consumptions were duplicated with an error of 5 per cent or less. The curves show that the Group A rats increased 15 to 20 gm. in weight and then came into another approximate weight maintenance at the higher level. At the end of 48 days, 2 gm. per day of non-protein-containing diet (Diet E) were given additionally to each of these rats (Period III-IV). Their body weights increased immediately, thus showing that the restriction which had been imposed upon them was one primarily of energy. The same type of experiment was repeated with the Group B animals. The food consumption of the last 24 days (Period II-III) of the 48 day experiment was used as a basis for an additional 24 day feeding (Period III-V) of the deficient diet with the 500 mg. supplement of tryptophane. The body weights increased as before—about 5 to 10 gm., to a new maintenance level. And again, addition of the energy diet caused a rapid increase in the body weights (Period V-VI). It appears, therefore, that a rat eating *ad libitum* of a diet limited in one essential factor (as to cause approximate weight maintenance)

ingests an amount of energy which is just about the quantity that can be used advantageously along with the amount available of the limiting factor, and very little more than this. If the limiting factor in the deficient diet were raised to a higher level so that the control animals were growing at a fair rate, then it might be that the test animals restricted to the food consumption of the controls would show a more distinct and continued response to further additions of the factor in question. Even if that were the case, a considerable portion of the method's sensitiveness would be lost in having to distinguish between two rates of growth rather than between growth and maintenance.

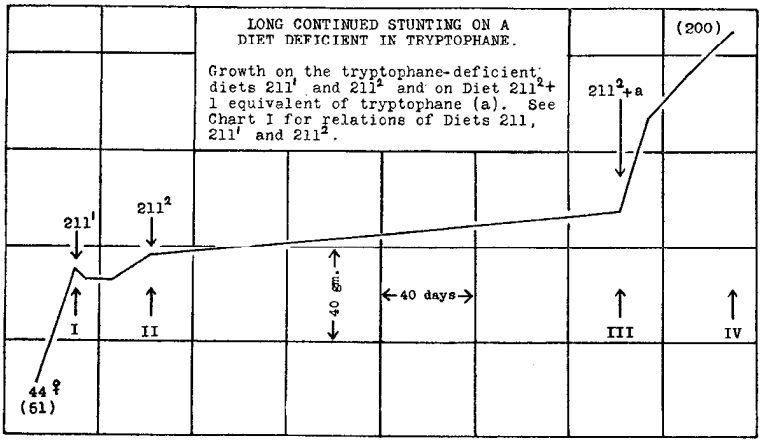


CHART VII.

A Case of Long Continued Stunting.

In the course of this study it was debated whether an animal confined for an extremely long period to a tryptophane-deficient diet of the type employed here—containing 15 per cent of free amino acids—would grow at a fair rate after inclusion of more tryptophane. Chart VII shows the growth of Rat 44 under such conditions. The animal's weight at the beginning of the stunting was 100 gm.; 232 days later, it was 125 gm. Inclusion of 250 mg. of tryptophane per 100 gm. of the diet now resulted in a 75 gm. body weight increment in 44 days. During Period II-III (see Chart VII) covering 200 days, the body weight never varied

as much as 2 gm. from a straight line drawn through all of the graphed body weight values. When the animal had reached the weight of 200 gm., a necropsy revealed large stores of fat; the internal organs appeared perfectly normal. The kidneys together weighed 1.3 gm. The two photographs (Fig. I) depict the appearance of the animal at the end of the stunting period and again at the end of the final growth period. These results are of interest when compared with those secured by Osborne and Mendel (1915) with Rat 2161 which was stunted upon the unhydrolyzed protein zein. The pictures of Rat 2161 are quite comparable to those of Rat 44. Particular attention is called to the hair coats in the two cases. Outstanding harm does not necessarily follow

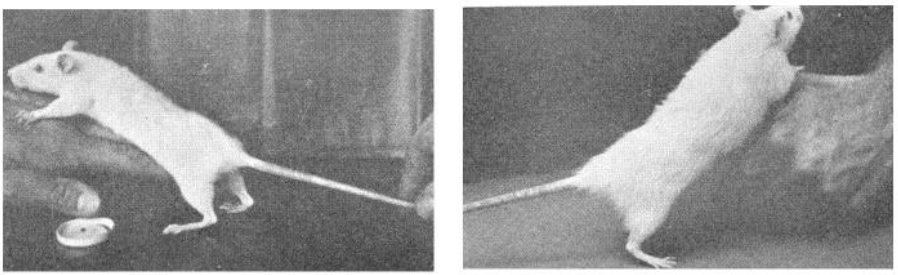


FIG. 1. Photographs of Rat 44 (left) after long continued stunting on a tryptophane-deficient diet, and (right) after a period of growth on the deficient diet supplemented with tryptophane. See points marked III and IV on Chart VII.

long continued stunting under the conditions herein described. In this connection may be recalled Osborne and Mendel's extensive demonstration of the rat's ability to resume normal growth after extremely long periods of stunting.

SUMMARY.

With one exception all the members of a tested series of tryptophane derivatives of physiological significance possesss no appreciable capacity to replace tryptophane in the diet.

Indole pyruvic acid, however, causes resumption of growth in animals deprived of tryptophane.

Data bearing on the relation of growth and food consumption are presented.

An instance of long continued stunting upon a tryptophane-deficient diet is described.

At the XIIIth International Physiological Congress at Boston, August, 1929, Berg, Rose, and Marvel reported a similar result with regard to the indole pyruvic acid; that is, they also have found that indole pyruvic acid serves as a biological substitute for tryptophane.

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