Stållberg-Stenhagen (1950) has synthesized (–)-21-methyltricosanoic acid ($[M]_D$, –13) by a route which shows that its methyl group is D-oriented in terms of the Fischer convention. The (+)-acid would therefore have $[M]_D$ +13, and would be the 21 L-methyl acid.

The (+)-*ante-iso* acids, the rotations of which are

collected in Table 1, are therefore L-methyl acids as shown by formula I.

SUMMARY

The (+)-*ante-iso*-alkanoic acids are represented by the Fischer projection formula I, and may be termed L-methyl-substituted acids.

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The Relation of Liver Kynureninase to Tryptophan Metabolism in Pyridoxine Deficiency

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The enzyme kynureninase splits alanine from the side chain of kynurenine and hydroxykynurenine to form anthranilic and hydroxyanthranilic acids, respectively (Kotake & Nakayama, 1941; Braunshtein & Goryochenkova, 1949; Wiss & Fuchs, 1950). The enzyme requires pyridoxal phosphate as coenzyme, and its activity in liver is decreased in pyridoxine deficiency (Braunshtein & Goryochenkova, 1949; Dalgliesh, Knox & Neuberger, 1951; Mason & Berg, 1952). The substrates of this enzyme, kynurenine and hydroxykynurenine, and their derivatives which retain the original carbon skeleton of the tryptophan side chain, accumulate in pyridoxine-deficient animals (Dalgliesh, 1951; 1952*a*). Therefore, the lack of pyridoxal phosphate, with its consequent decrease in kynureninase activity and accumulation of compounds usually removed by this enzyme, offers a qualitatively satisfactory explanation of the deranged tryptophan metabolism in pyridoxine-deficient animals.

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It is, however, necessary to show that, in pyridoxine deficiency, the rate of kynurenine formation exceeds that of kynurenine removal, before the observed reduction of kynureninase activity can be accepted as an adequate explanation for the excretion of kynurenine. The potential rates of kynurenine formation by the tryptophan peroxidase of rat liver have already been determined (Knox, 1951). The potential rates of kynurenine removal by kynureninase, in the livers of normal and pyridoxine-deficient rats, have now been determined for comparison. A new method of measuring the kynureninase activity was developed, and the properties of the enzyme were studied to ensure that this assay revealed the full potential activity of the enzyme. Measured under optimal conditions, the kynureninase activity of normal rat liver was higher than that previously determined (Braunshtein & Goryochenkova, 1949; Wiss & Hatz, 1949), and was, in fact, of a magnitude very similar to that of the tryptophan peroxidase. This similarity in the potential rates of kynurenine removal and formation under normal conditions is in keeping with the complete metabolism of tryptophan, without

accumulation of kynurenine, in normal rats. In livers of pyridoxine-deficient rats, the potential kynureninase activity was found to be decreased to about half, and was thus less than that of the tryptophan peroxidase. The ability to remove kynurenine in pyridoxine deficiency was therefore sufficiently depressed to account for the accumulation of some kynurenine and its derivatives by deficient animals on an unsupplemented diet. However, the very great accumulation of kynurenine and its derivatives, found when extra tryptophan is administered to pyridoxine-deficient animals, must be attributed largely to superimposition of the greatly increased kynurenine-forming potential, caused by the adaptive increase in the tryptophan peroxidase (Knox, 1951), upon the limited activity of the kynureninase.

EXPERIMENTAL

Preparation of enzyme. Rat livers were disintegrated in 2 vol. of cold 0.14M-KCl with a Waring Blendor and centrifuged in the cold. The supernatant (S1) was acidified at 0° with 0.5M-acetic acid to pH 4.8 (approx. 8 ml. of acid/100 ml. of S1), promptly centrifuged in the cold and the supernatant (S2) neutralized to pH 6.5. Cold ethanol was then added slowly to a final concentration of 45% (v/v) at -5°. The resulting precipitate (A1) was collected by centrifugation at -5° and redissolved in half the original volume of ice-cold 0.14M-KCl. The active fraction, which precipitated between 0.33 and 0.6 saturation with $(\text{NH}_4)_2\text{SO}_4$ (AS2), was dissolved in a small volume of water and dialysed against running water at 5° for 12 hr. The protein content of each fraction was determined from the optical densities at 260 and 280 m μ . (Warburg & Christian, 1941). The procedure yielded about 50% of the initial activity in the final fraction (AS2), and increased the activity/mg. of protein about 20-fold. The fractions retained their activities for several days at 2°.

Assay. An amount of kynureninase which would split not more than about 0.5 μ mole of kynurenine/hr. was pre-incubated at 38° for 15 min. with 20 μ g. of pyridoxal phosphate in a total volume of 1.0 ml. at a final concentration of 0.04M-borate or phosphate buffer, pH 8.0. In some experiments, as noted, 10 μ moles of pyruvate or α -ketoglutarate were included in the reaction mixture to stimulate kynurenic acid formation. Experimental and blank tubes then received 0.2 ml. of 7.5×10^{-3} M-L-kynurenine and 0.2 ml. water, respectively. One pair was immediately deproteinized by addition of 5 ml. of 1% (w/v) boric acid in ethanol, and a second pair after 40 min. incubation, for determination of the initial and final kynurenine concentration. Kynurenine was completely recovered by the boric acid-ethanol deproteinization, and its absorption spectrum in the neutral filtrate was not affected by slight changes in the pH of the reaction mixture. The filtrates and their appropriate blanks were read at 365 m μ . in a Beckman model DU spectrophotometer, and the kynurenine concentrations were calculated from the extinction coefficient of kynurenine in this medium (ϵ at 365 m μ . = 5150). The absorptions of kynurenic and anthranilic acids at 365 m μ . were less than 3% of that of kynurenine, and were neglected

in the usual calculations of kynurenine concentration. The validity of this determination of kynurenine was confirmed by the results obtained with the diazotization procedure given below. The enzyme activity was expressed as the μ moles of kynurenine utilized/ml. of enzyme or mg. protein/hr.

Paper-chromatographic identification of reaction products. Alanine, kynurenic acid, anthranilic acid and residual kynurenine were identified in the reaction mixture by drying 0.5–1.0 ml. of the boric acid-ethanol filtrate on to a single spot on filter-paper sheets, which were then chromatographed in butanol-acetic acid-water according to the procedure of Dalglish (1952a).

Determination of kynurenine and anthranilic acids by diazotization. Diazotization of the reaction mixtures before and after ether extraction was used to confirm the spectrophotometric determinations of kynurenine and anthranilic acid. Anthranilic acid was quantitatively removed from 5% trichloroacetic acid filtrates by two extractions with 2 vol. of ether, and all the kynurenine was left in the aqueous phase. Extracted and unextracted filtrates and standard solutions of each compound were determined at the same time by the method of Bratton & Marshall (1939), with an extra 1 ml. N-HCl added to each tube. Diazotization was done at 0° and the colours were read exactly 90 min. later at 550 m μ . The colour formed in the extracted filtrate, and the extra colour found in the unextracted filtrate, were calculated from the appropriate standards as kynurenine and anthranilic acid, respectively.

Spectrophotometric determination of kynurenic acid. From the observed optical densities of the reaction filtrates at 365, 330 and 310 m μ ., the percentage of kynurenic acid present could be calculated by the ratio method of Perry, Sutherland & Hadden (1950). This calculation, solved by the method of Crout (1941), also gave independent measures of the only other components absorbing in this region, namely kynurenine and anthranilic acid; the results agreed acceptably with the values determined by diazotization (Table 1). The adjusted matrix used in the calculations was constructed from the molar absorption coefficients: 5150, 2060, 894; 151, 8850, 3990; and 98, 2290, 3280; for kynurenine, kynurenic acid and anthranilic acid, at 365, 330 and 310 m μ ., respectively. Only freshly dissolved solutions of kynurenine, free from the adventitious absorption developing with age in the region of 310 m μ ., could be used as the substrate without introducing error into these determinations.

Compounds. N^α-Acetyl-DL-kynurenine and DL-kynurenine (Dalglish, 1952b) were provided by Dr C. E. Dalglish, and α -diketo- γ -(*o*-nitrophenyl)-butyric and α -diketo- γ -phenylbutyric acids were provided by Dr A. Neuberger. The 3-hydroxy-DL-kynurenine (Butenandt, 1949) was a gift to Dr A. Neuberger from Prof. A. Butenandt, and the pyridoxal phosphate was a gift from Dr W. W. Umbreit. The disodium salt of α -diketovaleric acid was prepared by the method of Meister & Greenstein (1948). L-Kynurenine was isolated, after feeding L-tryptophan, from rabbit urine (Butenandt, Weidel, Weichert & Derjugin, 1943).

RESULTS

Reaction products of kynurenine. Kynurenine disappeared primarily through the kynureninase reaction during anaerobic incubation with either crude or purified liver preparations. Only an-

Table 1. *The amounts of anthranilic and kynurenic acids formed from kynurenine by liver kynureninase preparations*

(Conditions as given in text. The products were determined by the spectrophotometric ratio method in all experiments, and in two of the experiments (the values in brackets) the kynurenine and anthranilic acid were also determined by diazotization. Pyruvate 10 μ moles, where used.)

Enzyme	(1) Kynurenine disappearance (μ moles)	(2) Anthranilic acid formed (μ moles)	(3) Kynurenic acid formed (μ moles)	Kynurenine (1) accounted for as (2) + (3) (%)
Dialysed S1	0.62 (0.59)	0.48 (0.44)	0.11 (0.15)*	95 —
Dialysed S1 + pyruvate	0.86	0.52	0.32	98
AS2	0.86	0.76	0.10	100
AS2 + pyruvate	0.88 (0.83)	0.62 (0.58)	0.24 (0.25)*	98 —

* Determination by difference from diazo values.

Table 2. *Relation of acylpyruvase and kynurenic acid-forming activities to the activity of liver kynureninase on purification*

(Fractions prepared and assayed as given in text. All activities were calculated as μ moles of substrate/hr. Acylpyruvase assayed under (1) with α -diketo- γ -(*o*-nitrophenyl)-butyrate; under (2) with α -diketo- γ -phenylbutyrate. Purified acylpyruvase prepared by the method of Connors & Stotz (1949).)

Fraction	Total kynureninase activities (μ moles/ml./ hr. $\times$ ml.)	Kynureninase purity (μ moles/mg. protein/hr.)	Acylpyruvase/kynureninase (molar ratio of activities)		Kynurenic acid formation (% of kynurenine disappeared)	
			(1)	(2)	Without pyruvate	With pyruvate
S1	258	0.015	2.5	7.5	18	39
S2	175	0.030	1.7	5.1	8	24
A1	153	0.074	0.2	0.6	2	18
AS2	126	0.303	0.05	0.15	9	28
AS2 + acylpyruvase	125	—	8.4	25.0	11	22

thranilic acid and alanine, the products of the kynureninase reaction, and a small amount of kynurenic acid were detectable chromatographically at the end of the reaction. The balance between the amount of kynurenine which reacted and the sum of the aromatic products formed, as determined by two different methods (Table 1), confirmed the essential absence of other reaction products. In the average preparation 80–90% of the kynurenine which disappeared was converted to anthranilic acid by kynureninase, while the rest was accounted for as kynurenic acid.

The invariable formation of kynurenic acid raised slightly the apparent kynureninase activity as measured by kynurenine disappearance. The proportion reacting in this way was small and was fairly constant in different preparations, even after partial purification (Table 2), but could be doubled by the presence of pyruvate or α -ketoglutarate (Tables 1 and 2). The preliminary dialysis of crude preparations to be assayed for kynureninase activity minimized this reaction.

No evidence could be obtained that the kynurenic acid arose from the related α -diketo acid, formed by a separate transamination of kynurenine or as a possible intermediate in the kynureninase reaction

itself (Dalglish *et al.* 1951). Acylpyruvase, which could hydrolyse this hypothetical intermediate to anthranilic acid and pyruvate (Meister & Greenstein, 1948; Connors & Stotz, 1949), did not participate in the kynureninase reaction, nor when added in excess did it decrease kynurenic acid formation (Table 2). Acylpyruvase was assayed with α -diketo- γ -phenylbutyrate and α -diketo- γ -(*o*-nitrophenyl)-butyrate as substrates, in lieu of the unknown but similar derivative of kynurenine, α -diketo- γ -(*o*-aminophenyl)-butyrate. The rates of hydrolysis of these two compounds by purified acylpyruvase (Connors & Stotz, 1949) were 15 and 5%, respectively, of that of α -diketovalerate. The ratio of acylpyruvase to kynureninase activities, both calculated as moles of substrate split per unit time, in the kynureninase preparations at various stages of purification, are given in Table 2. The initially higher activity of acylpyruvase was readily decreased by purification to well below that of kynureninase without affecting kynurenine disappearance or the formation of either anthranilic or kynurenic acids.

Substrate concentration. The effect of kynurenine concentration on the rate of its removal by a liver kynureninase preparation has been plotted in Fig. 1

according to the method of Lineweaver & Burk (1934). The enzyme was half-saturated (K_s) at 4×10^{-4} M-L-kynurenine. It may be significant that the enzyme tryptophan peroxidase, which forms kynurenine in liver, had an identical K_s (Knox & Mehler, 1950). The value for the analogous bacterial kynureninase was considerably lower (3.9×10^{-5} M) (Hayaishi & Stanier, 1952). The substrate concentrations used in previous assays of animal kynureninase ($< 1 \times 10^{-3}$ M) were therefore initially sub-optimal, and the progressive slowing down of the

Fig. 1. The effect of kynurenine concentration (S) on the rate of the kynureninase reaction (V). 40 min. incubation of 0.5 ml. dialysed liver supernatant. (V = μ moles kynurenine utilized/40 min.; S = initial concentration of L-kynurenine, $M \times 10^{-3}$; K_s = dissociation constant of enzyme-substrate complex.)

reaction, as the concentration fell during the long incubations, was mainly responsible for the low activities reported (Kotake & Nakayama, 1941; Wiss & Hatz, 1949), as was recognized by Braunshtein & Goryochenkova (1949). Halving the already inadequate L-kynurenine concentration, as when DL-kynurenine was substituted as substrate (Wiss, 1949), further reduces the kynureninase activity in the absence of any inhibition of the reaction by the D-component. Only L-kynurenine was used as substrate in these assays.

From the data in Fig. 1 it was calculated that the initial rate with 1.25×10^{-3} M-L-kynurenine, which was convenient to use, and the final rate after the reaction of 0.5 μ moles (i.e. with 0.83×10^{-3} M-kynurenine) would be 74 and 66 % of the maximal kynureninase reaction velocity (V_m). The slowing of the reaction to this extent during the assay could not be detected by the difference measurements made, and these concentration limits were chosen for the assay of liver kynureninase. The maximal velocity with unlimited substrate could not be

determined accurately by this method because of the intense absorption of kynurenine, but the V_m was calculated to be about 1.4 times the rate actually measured. The values reported have not, however, been corrected by this factor.

pH Optimum. The maximal activity of rat liver kynureninase occurred at pH 8.0, which was nearer the optimum of bacterial kynureninase (pH 8.5) than the values previously reported (7.4 and 7.5) (Hayaishi & Stanier, 1952; Kotake & Nakayama, 1941; Braunshtein & Goryochenkova, 1949; Wiss, 1949). The rates at pH 6.5, 7.0, 7.4 and 8.5 were 25, 43, 81 and 77 %, respectively, of the rate at pH 8.0. The spontaneous decomposition of kynurenine during incubation at pH values above 8.5 precluded measurements at more alkaline reactions. The reaction occurred equally well in phosphate and borate buffers, and no requirement for phosphate could be detected (Wiss, 1949).

Coenzyme. Even in an undialysed liver supernatant the kynureninase was apparently already partially resolved, since addition of pyridoxal phosphate increased the kynureninase activity by about 25 %. However, dialysis alone did not increase the degree of resolution, so the activities found in homogenates after preliminary dialysis were assumed to be measures of the enzyme activity *in vivo*. One precipitation with ammonium sulphate followed by dialysis regularly produced about 70 % resolution of the enzyme.

Maximal restoration of the activity of the partially resolved enzyme was obtained by pre-incubation with its coenzyme for 15 min. before the reaction. Thus a resolved enzyme preparation showed only 31 % activity without pyridoxal phosphate, and 65 % activity with pyridoxal phosphate added at the start of the reaction, compared with 100 % activity when first pre-incubated for 15 min. with its coenzyme. Pyridoxal phosphate in 20 μ g. amounts was in moderate excess over the requirement of all enzymes tested.

Specificity. Only the L-form of kynurenine was split by the enzyme. When 5.0 μ moles of L-kynurenine and of DL-kynurenine were separately allowed to react to completion with excess of enzyme, 4.53 and 4.83 μ moles of L-kynurenine and 2.46 and 2.47 μ moles of DL-kynurenine, respectively, had reacted after 60 and 100 min. When equal concentrations of L-kynurenine were tested, as L-kynurenine itself and as twice this concentration of DL-kynurenine to correct for the effect of substrate concentration upon the rate of reaction, no inhibition by the inactive D component was observed.

Only sufficient 3-hydroxy-DL-kynurenine was available to ascertain that it was split by kynureninase slightly more rapidly than was DL-kynurenine under the same conditions. The absorption spectrum of the products resembled that of a mixture of

3-hydroxyanthranilic and xanthurenic (4:8-dihydroxyquinolic-2-carboxylic) acids, but the products were not definitely identified. Both kynurenine and hydroxykynurenine accumulated in pyridoxine-deficient animals (Dalglish, 1952*a*), and since both reacted similarly with the enzyme, either or both could be the normal substrates of kynureninase. The reaction studied, therefore, represents the physiological mechanism by which both can be definitely metabolized through loss of their side-chains. The quantitative aspects of the reaction are probably similar with both compounds, but have in the present work been definitely established only for kynurenine.

The other side-chain-retaining metabolites identified as accumulating in pyridoxine-deficient animals (Dalglish, 1952*a*), kynurenic and xanthurenic acids and *N*^α-acetylkynurenine, were not substrates for kynureninase. *N*^α-Acetyl-DL-kynurenine was deacetylated, however, during incubation with the kynureninase preparations by a potent aliphatic deacetylase present (Bray, James, Raffan, Ryman & Thorpe, 1949), and the product could then be split by kynureninase. Purification of the kynureninase preparations did not eliminate this deacetylase, but its relative activity was decreased. The rate of hydrolysis of acetylkynurenine in the kynureninase reaction was consequently diminished as the deacetylase was decreased in the S1, A1 and AS2 stages of purification to 44, 32 and 28 % of the rate of L-kynurenine hydrolysis.

Assay of kynureninase. From the properties of kynureninase described in the preceding sections, nearly optimal conditions for the enzyme assay were chosen, as given in the Methods. As calculated from the effect of the substrate concentration on the reaction rate, the reaction was substantially linear under these conditions until about 0.5 μmoles of kynurenine had reacted. All assays were made within this limit, and the reaction was taken to be proportional to time and to the enzyme concentration (Fig. 2). Serial measurements of the disappearance of larger amounts of kynurenine during longer incubation periods showed, however, that the reaction was then first order.

The amount of kynurenine which reacted per unit time in this assay can be taken to represent the potential metabolic ability of the liver preparation examined to remove the original tryptophan side chain from the only compounds which can react in this way, kynurenine and hydroxykynurenine. The values obtained were subject to the qualifications that they were 10–20 % too high due to the formation of kynurenic acid being included, that they might be 1.4 times higher with unlimited substrate concentrations and that they might be slightly different if hydroxykynurenine were the more important substrate under physiological conditions.

Rat kidney, when tested for kynureninase activity by the same assay, was only about 10 % as active per unit weight as liver. Therefore both the formation of kynurenine and its removal occurred predominantly in the liver, and the rates of these reactions in liver will be of primary significance in determining the magnitude of tryptophan metabolism observed in whole animals.

Fig. 2. The relation of kynurenine disappearance to enzyme concentration and to time of incubation. Dialysed supernatant fraction of liver (S1) assayed as given under Methods for 20 min. (●); 40 min. (O); and 60 min. (x).

Levels of kynureninase in rat livers. Equal numbers of male and female rats were maintained for 3–6 months on the pyridoxine-deficient diet of Dalglish (1952*a*). Controls on the same diet with added pyridoxine were found to have the same enzyme activity as animals on a stock diet, and are not separately reported. No sex difference in the enzyme activity was observed. The assays were conducted in the system described, using 0.5 ml. of a freshly prepared 33 % (w/v) whole liver homogenate which had been dialysed for 2 hr. against running tap water. The activities/g. dry wt. of liver/hr., with and without added pyridoxal phosphate, found in the normal and the pyridoxine-deficient animals are given in Table 3. The kynureninase activity in the deficient livers was about half that of the normals, and since the livers of the deficient animals were smaller, their absolute kynureninase activity was much less than that of normal animals. With added pyridoxal phosphate the activity of the deficient preparations was restored nearly to normal, indicating that the enzyme protein was maintained during the deficiency.

Table 3. *Levels of liver kynureninase in normal and pyridoxine-deficient rats*

(Kynureninase determinations as described in text with and without addition of pyridoxal phosphate to liver homogenates. Activity expressed as μ moles kynurenine disappearing/hr./g. dry wt. Pyridoxal phosphate 20 μ g., where used.)

	Normal rats		Pyridoxine-deficient rats	
	No addition	Pyridoxal phosphate added	No addition	Pyridoxal phosphate added
No. of animals	10	40	7	5
Average activity	11.1	14.3	5.9	12.7
S.E.M.	± 0.80	± 0.03	± 1.25	± 0.71

The kynureninase activity was measured in the same way in the livers of two normal rats 6 hr. after they had been given intraperitoneally either 1 or 2 m-moles of DL-kynurenine. The activities found were in the normal range (12.6 and 15.7 μ moles/g. dry wt./hr.). A third rat 6 hr. after receiving 2 m-moles of DL-tryptophan also had a normal kynureninase activity. This treatment increased the activity of the tryptophan peroxidase in the liver from the normal value of 9.3 (Knox, 1951) to 30.5. The kynureninase could apparently not be adapted by its substrate, nor successively by tryptophan, as can the kynurenine-forming enzyme, tryptophan peroxidase.

DISCUSSION

The rates of kynureninase activity in liver, in comparison with the previously determined rates of kynurenine formation, show that removal of the original tryptophan side chain by kynureninase can become the limiting step in tryptophan metabolism. When this occurs, it permits the accumulation of kynurenine and hydroxykynurenine, and their secondary conversion to the other metabolites containing this side chain which are excreted by pyridoxine-deficient animals. This interpretation rests upon the justifiable assumption that the full potential rates of enzymes measured *in vitro* bear some relation to the rates at which they can react *in vivo*. Under the condition of these experiments, the major reaction of kynurenine occurred in liver, where it is also formed, and consisted of conversion to anthranilic acid and alanine. The oxidation of kynurenine to hydroxykynurenine was not investigated, since either or both of these compounds may be the normal substrate of kynureninase, and the reactions of both are presumably very similar. The other tryptophan metabolites tested did not lose their side chain except secondarily by this reaction. In association with the kynureninase reaction, as measured, small amounts of kynurenic acid were formed. Its formation was largely dependent upon the presence of an α -keto acid such as pyruvate, but in the absence of pyruvate it was not a quantitatively important reaction and did not seriously affect the measurement of kynureninase

activity. Only one enzyme was apparently involved in the kynureninase reaction itself and it was assayed under its optimal conditions, except that its maximal velocity with unlimited substrate concentrations was calculated to be about 1.4 times that actually measured. Subject to the qualifications mentioned, the kynureninase activity may be taken as a measure of the potential rate at which the side chain of tryptophan metabolites can be removed.

In the livers of normal and pyridoxine-deficient rats not supplemented with extra coenzyme the kynureninase activity was 11.1 and 5.9 μ moles/g. dry wt./hr., respectively. The rate at which kynurenine was formed from L-tryptophan in normal rat liver, and which was not changed in pyridoxine deficiency (Braunshtein & Goryochenkova, 1949), averaged 9.3 μ moles/g. dry wt./hr. (Knox, 1951). This comparison shows that kynurenine can potentially be formed and removed in normal livers at about the same rates. Halving the kynureninase activity, which occurred in the pyridoxine-deficient animals, might therefore result in the accumulation of a little kynurenine, and secondary conversion of it to other derivatives, to produce the small amounts of tryptophan metabolites excreted by pyridoxine-deficient animals not given supplementary tryptophan.

With the administration of extra tryptophan to animals, their tryptophan peroxidase activity can be raised at least tenfold by specific enzyme induction (adaptation), and under other conditions by hormonal action (Knox & Mehler, 1951; Knox, 1951), to increase greatly the kynurenine-forming potential. The kynureninase activity, however, was not found to be altered adaptively by administration of either kynurenine or tryptophan. The unchanged potential for removing kynurenine, together with the increased ability to produce it, can explain the excretion of kynurenine and its derivatives which occurs when even normal animals are given large doses of tryptophan (Porter, Stoerk & Silber, 1951). The still greater excretion of kynurenine and its derivatives seen when tryptophan is administered to pyridoxine-deficient animals must be attributed largely to this increased formation of kynurenine by the tryptophan-adapted

tryptophan peroxidase, superimposed upon the slower removal of kynurenine by kynureninase lacking an adequate concentration of its coenzyme.

SUMMARY

1. Under optimal conditions, the average kynureninase activity of normal rat liver was $11.1 \mu\text{moles L-kynurenine removed/g. dry wt./hr.}$ The activity in pyridoxine-deficient rats was decreased by lack of the coenzyme to $5.9 \mu\text{moles/g. dry wt./hr.}$ The kynureninase was not increased adaptively in the animals by administration of kynurenine or tryptophan.

2. For comparison, kynurenine can be formed by the tryptophan peroxidase in normal rat liver at an

average rate of $9.3 \mu\text{moles/g. dry wt./hr.}$, and this rate can be adaptively increased tenfold by tryptophan administration to the animal.

3. The abnormal accumulation of tryptophan metabolites can therefore be attributed to a relative inadequacy of kynurenine removal, due to a decrease of kynureninase through lack of its coenzyme, or to an adaptive increase of the kynurenine-forming enzyme, tryptophan peroxidase, or to both. These mechanisms can account for the marked accumulation of metabolites in even normal animals given supplemental tryptophan, as well as in pyridoxine-deficient animals.

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Studies on the Polarography of Proteins

1. THE RELATION OF WAVE HEIGHTS TO PROTEIN CONCENTRATION AND THE ORIGIN OF WAVE III

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When a protein is added to a polarographic cell containing a buffer, the polarogram consists of a single large wave which begins to rise at about -1.8 V. with reference to the saturated calomel electrode (s.c.e.). This wave was identified as a protein wave and its characteristics were described by Heyrovský & Babička (1930); it was called the prenatium wave by Herles & Vančura (1932). Brdička (1933a) accidentally discovered a second

protein effect. If the electrolyte contained ammonia and either bivalent or trivalent cobalt in addition to the protein, the reduction wave of bivalent cobalt was followed by two waves (frequently referred to as a double wave) which were due to the protein (Fig. 1). These last waves will be referred to as waves I and II respectively. He found also that, of a series of amino-acids tested, only cystine or cysteine produced a similar effect, for in an ammoniacal