

R71-104

Reprinted from

Computer Simulation and Analysis of Tryptophan Metabolism via Kynurenine Pathway in Liver

F. HEINMETS

Pioneering Research Laboratory, U.S. Army Natick Laboratories,
Natick, Mass. 01760, U.S.A.

(Received 29 January 1971 and in revised form 5 April 1971)

Abstract—Tryptophan metabolism via the kynurenine pathway has been studied through the simulation of a model system on an analog computer. Experiments pertaining to the coenzyme effect of pyridoxal on metabolite distribution were performed under a variety of conditions. The effects of regulatory feedback on some enzyme activities and metabolite concentrations were explored. Tryptophan-load experiments were

TECHNICAL LIBRARY
U. S. ARMY
NATICK LABORATORIES
NATICK, MASS. 01760



PERGAMON PRESS
OXFORD NEW YORK LONDON PARIS

Vault

Computer Simulation and Analysis of Tryptophan Metabolism via Kynurenine Pathway in Liver

F. HEINMETS

Pioneering Research Laboratory, U.S. Army Natick Laboratories,
Natick, Mass. 01760, U.S.A.

(Received 29 January 1971 and in revised form 5 April 1971)

Abstract—Tryptophan metabolism via the kynurenine pathway has been studied through the simulation of a model system on an analog computer. Experiments pertaining to the coenzyme effect of pyridoxal on metabolite distribution were performed under a variety of conditions. The effects of regulatory feedback on some enzyme activities and metabolite concentrations were explored. Tryptophan-load experiments were performed. The conclusion is drawn that model systems established on currently available information on tryptophan metabolism in liver are not adequate to interpret various abnormal distribution patterns of metabolites under conditions of disease. Also, current clinical measurements on a variety of metabolic intermediates do not yield significant information on the mechanism of disease.

TRYPTOPHAN is one of the key amino acids in mammalian metabolism. Not only does it serve as a building unit for enzyme synthesis, but its derivatives have multiple roles in various regulatory processes. Its principal degrading enzymes, tryptophan pyrolase in the liver and tryptophan hydroxylase in the brain, are both inducible by corticosteroids.^(1,2) We have reviewed these inductive and regulatory processes elsewhere and have carried out computer simulation experiments.⁽³⁾ Furthermore, tryptophan metabolism and its cyclic behavior in the pineal gland have also been the subject of model development and computer analysis.⁽⁴⁾ Here we shall carry out a computer analysis of tryptophan metabolism via the kynurenine pathway in liver.

Faulty tryptophan metabolism in liver has been associated with a variety of chronic diseases, and extensive clinical research has been performed on human subjects.⁽⁵⁾ In addition, tryptophan metabolism is affected by a nutritional deficiency of pyridoxine,⁽⁶⁾ since this coenzyme participates in several enzyme reactions in the kynurenine pathway. Therefore, great care had to be taken to separate the modifications of tryptophan metabolism arising via dietary factors from metabolic disorders inherent in the system. ALTMAN and GREENGARD⁽⁶⁾ carried out the first extensive studies on human subjects dealing with pyridoxal deficiency and with the effect of hydrocortisone administration on tryptophan metabolism. They found that hydrocortisone caused two- to fourfold increases in the level of tryptophan pyrolase and in the amount of urinary kynurenine. In nutritionally normal subjects, there was a direct correlation between the activity of tryptophan pyrolase and the amount of urinary kynurenine. Their results suggest that in a variety of diseases the increased urinary excretion of kynurenine and its metabolites may be caused by increased oxidation of tryptophan. Urinary excretion has been the principal source for measuring the tryptophan metabolic products, and the question has been raised whether this approach is adequate. Some evidence suggests that measurements should also be performed on the

metabolite levels in the serum.⁽⁷⁾ There are indications that renal clearance may be a factor in some diseases and therefore urinary measurements are not adequate.

Tryptophan metabolism and its abnormalities have aroused widespread interest among a variety of investigators. For example, it occupies considerable space in some symposia (e.g. Vitamin B₆ in Metabolism of the Nervous System, New York Academy of Sciences, 30 September 1969). We shall not attempt to review this subject here since we have restricted our objective to a very limited problem, as follows: A great many experiments using the tryptophan-loading technique have been carried out on hospitalized human subjects with various diseases. These experiments are expensive and sometimes exert considerable stress on the subject. Since a great deal of clinical and laboratory specimen handling as well as a large number of measurements is required, the following questions can be posed:

(1) What is an adequate number of urinary and other measurements of the intermediates of the kynurenine pathway to resolve the "abnormality spectrum"; and can one draw far-reaching conclusions from a limited number of experiments?

(2) How can metabolic disorders and nutritional abnormalities which may occur at the same time be separated?

(3) Can one determine a critical set of measurements on the patient for diagnosing a disease?

Many other questions could be asked with a view to optimizing the experimental procedures. We do not propose to answer these questions here. However, we consider that the rational basis for planning experiments is knowledge about the kinetics of the metabolic pathways and about the factors which influence it. Therefore, we decided to study tryptophan metabolism by means of a model system and to carry out various simulated laboratory experiments on the computer, in order that we might learn more about the behavior of the metabolic system under a variety of conditions. Insight into the kinetic and other properties of the system shall lead to better planning of exploratory diagnosis and should help in drawing conclusions from the experiments. The computer-simulation approach seems especially applicable, since tryptophan metabolism via the kynurenine pathway is complex, entailing multiple branches containing several common enzymes.

While many attempts have been made to analyze the abnormalities of tryptophan metabolite patterns, in various diseases, very little success has been reported in associating specific enzymic-reaction schemes with particular abnormalities. On the contrary, abnormality patterns are difficult to interpret on the basis of current metabolic schemes of tryptophan degradation. This has been clearly demonstrated by more recent studies.^(8,9) What we expect to achieve by our computer-simulation experiments is to show the currently established properties of the tryptophan degradative pathway under various experimental conditions. Our principal aim is perhaps negative. It is to discourage statistical and other experiments that are expensive and stressful to the subjects, but do not have any inherent capacity to provide information meaningful to the resolution of the problem.

FORMULATION OF THE MODEL SYSTEM

Enzymic reactions in tryptophan metabolism in the liver are fairly well established, and reactions in a multiple branching pathway have been described and analyzed.^(5,7-9) Pyridoxine is the coenzyme which has the principal regulatory role in the pathway, and the activity of several enzymes depends on its presence in the system. While the pyridoxine

requirement for the kynureninases and transaminases has been clearly established, the effects of the coenzyme on the activities of these enzymes are not equal. The evidence is that kynureninase activity increases extensively, while transaminase activity is affected only moderately, in the presence of pyridoxine. These factors are taken into account during the modeling. Furthermore, there is some indication that kynureninase and hydroxykynureninase are two distinct enzymes, but the evidence is not conclusive.^(8,9) Similarly, there may be different transaminases. For current model-system analysis, these are fine points and they need not be considered here. While the enzymes in the kynurenine pathway are fairly well known, the regulatory interactions in this multibranched pathway have not been established. One may be quite certain that in a metabolic pathway as complex as that of tryptophan, degradation via kynurenine is indeed extensively regulated. However, data on this is currently not available. Therefore, we base our model on available information, since current experiments and diagnoses of abnormalities in tryptophan metabolism are planned and analyzed on such a basis.

The only regulatory feature which has been observed in *in vitro* experiments is the kynurenine inhibition of tryptophan pyrolase.⁽¹⁰⁾ This feature is included in the model. Furthermore, it is well established that tryptophan pyrolase synthesis is induced by corticosteroids^(1,2) and to a minor degree by tryptophan itself. This subject has been discussed in detail in a previous publication.⁽³⁾ Here we simulate only enzymic reactions in the tryptophan pathway, and the system has been formulated for such reactions. One can carry out computer experiments where there is an increase in the tryptophan pyrolase level. However, such effects can be produced simply by increasing the tryptophan input into the model system.

A general metabolic scheme for tryptophan metabolism is given in Figs. 1a and 1b, the corresponding symbols are given in Table 1. Only the pathway via tryptophan pyrolase (E_2^1) is considered here. Enzymic reaction steps are presented in Table 2. Since these are self-explanatory, no further discussion will begin. Perhaps it should be noted that tryptophan has a dual role in the beginning of the pathway: it acts as the input substrate, but it also serves as activator for tryptophan pyrolase.⁽²⁾ The flow equations in Table 2 are converted to differential equations (Table 3) and these are programmed for an analog computer. Details of such operations are not discussed here, since this has been done elsewhere.⁽¹¹⁾

RESULTS OF COMPUTER SIMULATION

Tryptophan pulse

The computer system is made operational by introducing tryptophan. The tryptophan concentration can be kept at a constant level or it may be given as a pulse (Fig. 2).

The effects of a tryptophan pulse on various metabolite concentrations are shown in Fig. 3. All metabolites are at normal ground level at the time (the first arrow) that tryptophan (s_2^1) is introduced into the system. Concentrations of all metabolites rise rapidly, and then decline when the s_2^1 pulse is discontinued until the basal levels are finally reached again.

THE EFFECT OF PYRIDOXAL ON METABOLITE LEVELS

It is well known that several enzymes require the presence of the coenzyme vitamin B₆ for their maximal activity. However, all enzymes are not equally affected. Here E_5^2 and

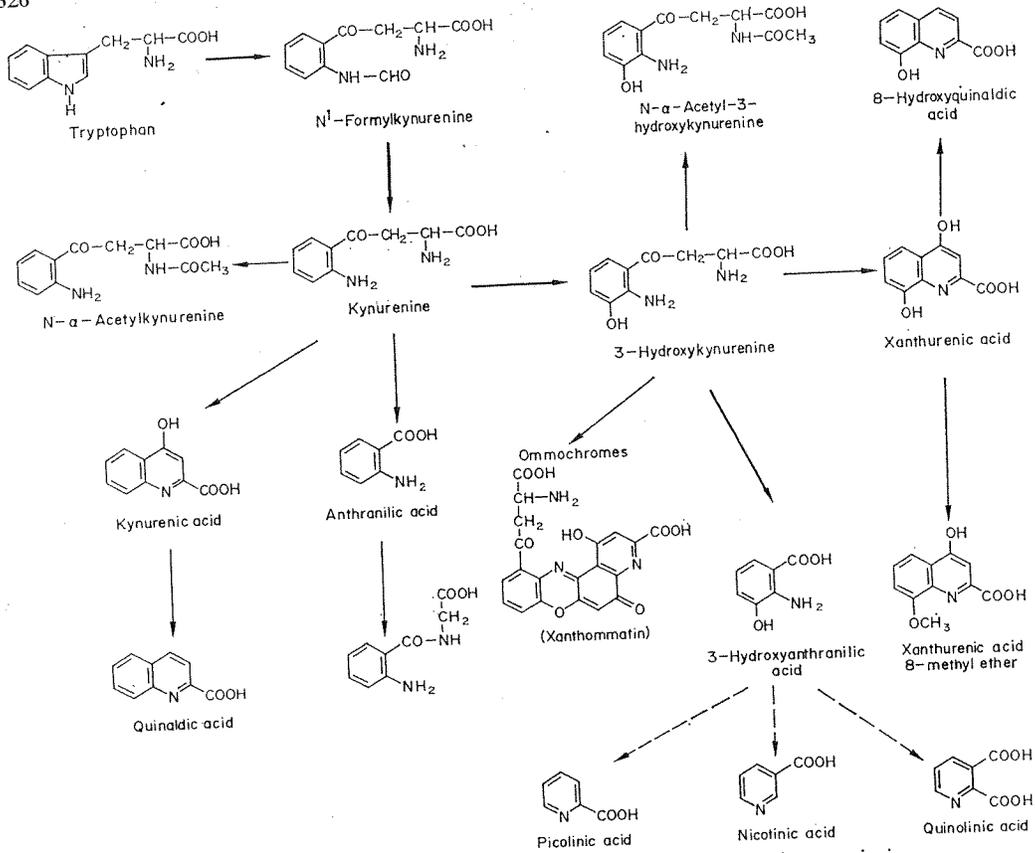


FIG. 1a. Kynurenine pathway for metabolic conversion of tryptophan to niacin.

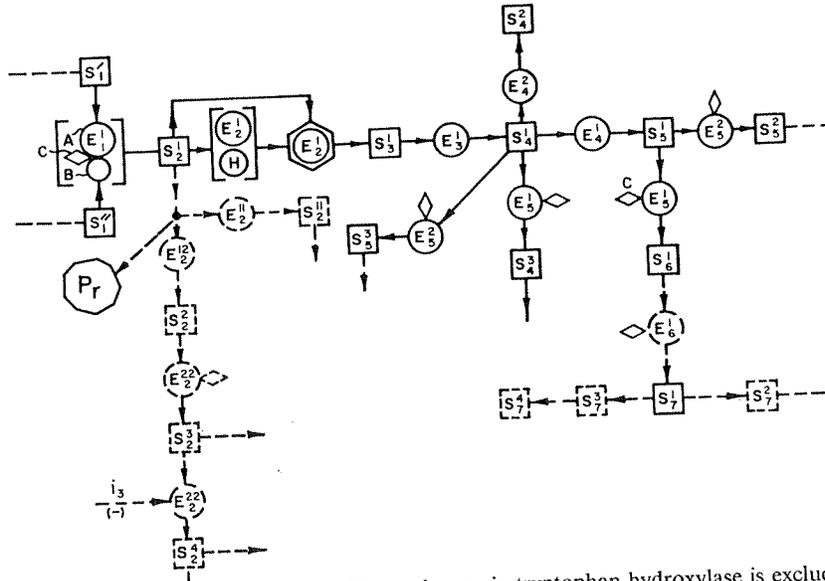


FIG. 1b. Metabolic scheme for tryptophan. The pathway via tryptophan hydroxylase is excluded from the analysis.

TABLE I. SYMBOLS AND FUNCTIONAL ENTITIES

P_r	—	Protein
s_1^1	—	Indole
s_1^{11}	—	Serine
s_2^1	—	Tryptophan
s_3^1	—	Formyl kynurenine
s_4^1	—	Kynurenine
s_5^1	—	3-Hydroxykynurenine
s_6^1	—	3-Hydroxyanthranilic acid
s_7^1	—	2-Acroleyl-3-amino fumarate
s_7^2	—	Acetyl coA
s_7^3	—	Quinolinic acid
s_7^4	—	NAD (nicotinamide adenine dinucleotide)
s_4^2	—	N- α -acetyl kynurenine
s_2^{11}	—	Tryptamine
s_4^3	—	Anthranilic acid
s_5^3	—	Kynurenic acid
s_2^2	—	5-Hydroxy- tryptophan
s_2^3	—	5-Hydroxy tryptamine (serotonin)
s_2^4	—	5-Hydroxy indole acetaldehyde
s_5^2	—	Xanthurenic acid
i_3	—	Inhibitor of E_2^{32}
A	—	Isoenzyme
B	—	Isoenzyme
C	—	Coenzyme (Vitamin B ₆)
E_1^1	—	Tryptophan synthetase + C
E_2^1	—	Tryptophan pyrolase
E_3^1	—	Kynurenine formylase
E_4^1	—	Kynurenine hydroxylase
E_5^1	—	Kynureninase + C
E_6^2	—	Kynurenine transaminase + C
E_6^1	—	3-Hydroxy anthranilic oxidase
E_2^{12}	—	Tryptophan 5-hydroxylase
E_2^{22}	—	5-Hydroxy tryptophan decarboxylase
E_2^{32}	—	Monoamine oxidase

*Indicates highly active state of the entity. See Tables 2 and 3.

TABLE 2. FLOW EQUATIONS

	Rate constant (k) subindex
1. $E_2^1 + s_2^1 \rightleftharpoons \dot{E}_2^1$	1, -1
1a. $E_2^1 + s_2^1 \rightarrow s_3^1 + E_2^1$	(k') 2
2. $\dot{E}_2^1 + s_2^1 \rightarrow s_3^1 + E_2^1$	2
3. $E_3^1 + s_3^1 \rightarrow s_4^1 + E_4^1$	3
4. $E_4^2 + s_4^1 \rightarrow s_4^2 + E_4^2$	4
4a. $s_4^1 + E_2^1 \rightleftharpoons [s_4^1 E_2^1]$	(k') 5, -5
5. $E_5^1 + s_4^1 \rightarrow s_4^3 + E_5^1$	5
6. $E_5^2 + s_4^1 \rightarrow s_5^3 + E_5^2$	6
7. $s_4^2 \rightarrow X$	7
7a. $s_4^1 \rightarrow s_4^{11}$	(k') 7
8. $s_5^3 \rightarrow X$	8
9. $E_4^1 + s_4^1 \rightarrow s_5^1 + E_4^1$	9
10. $E_5^2 + s_5^1 \rightarrow s_5^2 + E_5^2$	10
11. $E_5^2 + C \rightleftharpoons \dot{E}_5^2$	11, -11
12. $\dot{E}_5^2 + s_5^1 \rightarrow s_5^2 + \dot{E}_5^2$	12
13. $E_5^1 + C \rightleftharpoons \dot{E}_5^1$	13, -13
14. $\dot{E}_5^1 + s_4^1 \rightarrow s_4^3 + \dot{E}_5^1$	14
15. $\dot{E}_5^1 + s_5^1 \rightarrow s_6^1 + \dot{E}_5^1$	15
16. $\dot{E}_5^2 + s_4^1 \rightarrow s_5^3 + \dot{E}_5^2$	16
17. $E_5^1 + s_5^1 \rightarrow s_6^1 + E_5^1$	17
18. $E_6^1 + s_6^1 \rightarrow s_7^1 + E_6^1$	18
19. $E_6^1 + C \rightleftharpoons \dot{E}_6^1$	19, -19
20. $\dot{E}_6^1 + s_6^1 \rightarrow s_7^1 + \dot{E}_6^1$	20
21. $s_7^1 \rightarrow X$	21
22. $s_2^{11} \rightarrow X$	22
23. $s_4^3 \rightarrow X$	30
24. $s_5^2 \rightarrow X$	31
25. $s_2^1 + E_2^{11} \rightarrow s_2^{11} + E_2^{11}$	32
26. $s_2^3 \rightarrow X$	33

In equations 1a, 4a, 7a, rate constant is designated as k'; all others are k. These symbols appear in differential equations.

1.
2.
3.
4.
5.
6.
7.
8.
9.
10.
11.
12.
13.
14.
15.
16.
17.
18.
19.
Initial
Follow
(a) ;
(b) ;
(c) ;
(d) ;
Concer
cons

TABLE 3. DIFFERENTIAL EQUATIONS

1. $\dot{E}_2^1 = k_{-1} \dot{E}_2^{*1} - k_1 s_2^1 E_2^1 - k'_{5s_4^1} E_2^1 + k'_{-5} [s_4^1 E_2^1]$
2. $\frac{\dot{E}_2^1}{E_2^1} = k_1 s_2^1 E_2^1 - k_{-1} \dot{E}_2^{*1}$
3. $\dot{s}_3^1 = k_2 s_2^1 \dot{E}_2^1 - k_3 s_3^1 E_3^1 + k'_{2s_2^1} E_2^1$
4. $\dot{s}_4^1 = k_3 s_3^1 E_3^1 - k_4 s_4^1 E_4^2 - k_5 s_4^1 E_5^1 - k_6 s_4^1 E_5^2 - k_9 s_4^1 E_4^1 - k_{14} s_4^1 \dot{E}_6^{*1} - k_{16} s_4^1 \dot{E}_5^2 - k'_{5s_4^1} E_2^1 + k'_{-5} [s_4^1 E_2^1] - k'_{7s_4^1}$
5. $\dot{s}_4^2 = k_4 s_4^1 E_4^2 - k_7 s_4^2$
6. $\dot{s}_4^3 = k_5 s_4^1 E_5^1 + k_{14} s_4^1 \dot{E}_6^{*1} - k_{30} s_4^3$
7. $\dot{s}_5^3 = k_6 s_4^1 E_5^2 + k_{16} s_4^1 \dot{E}_5^2 - k_8 s_5^3$
8. $\dot{s}_5^1 = k_9 s_4^1 E_4^1 - k_{10} s_5^1 E_5^2 - k_{12} s_5^1 \dot{E}_5^2 - k_{15} s_5^1 \dot{E}_6^{*1} - k_{17} s_5^1 E_6^1$
9. $\dot{s}_5^2 = k_{10} s_5^1 E_5^2 + k_{12} s_5^1 \dot{E}_5^2 - k_{31} s_5^2$
10. $\dot{E}_5^2 = k_{-11} \dot{E}_6^{*2} - k_{11} C E_5^2$
11. $\frac{\dot{E}_5^2}{E_5^2} = -\dot{E}_5^2$
12. $\dot{E}_5^1 = k_{-13} \dot{E}_6^{*1} - k_{13} C E_5^1$
13. $\frac{\dot{E}_6^{*1}}{E_6^{*1}} = -\dot{E}_6^{*1}$
14. $\dot{s}_6^1 = k_{15} s_5^1 \dot{E}_6^{*1} + k_{17} s_5^1 E_6^1 - k_{18} s_6^1 E_6^1 - k_{22} s_6^1 \dot{E}_6^{*1}$
15. $\dot{s}_7^1 = k_{20} s_6^1 \dot{E}_6^{*1} + k_{19} s_6^1 E_6^1 - k_{21} s_7^1$
16. $\dot{E}_6^1 = k_{-16} \dot{E}_6^{*1} - K_{16} C E_6^1$
17. $\frac{\dot{E}_6^1}{E_6^1} = \dot{E}_6^1$
18. $[s_4^1 E_2^1] = k'_{5s_4^1} E_2^1 - k'_{-5} [s_4^1 E_2^1]$
19. $\dot{s}_4^{11} = k'_{7s_4^1}$

Initial conditions and values.

Following entities are considered to be constant during the kinetic process:

- (a) Substrate s_2^1 .
- (b) All enzyme concentrations.
- (c) Cofactors (coenzymes, inhibitors).
- (d) All rate constants.

Concentration of the following entities will be varied from initial values: $[s_2^1]_0$, $[C]_0$, $[i]_0$ and various rate constants as desired.

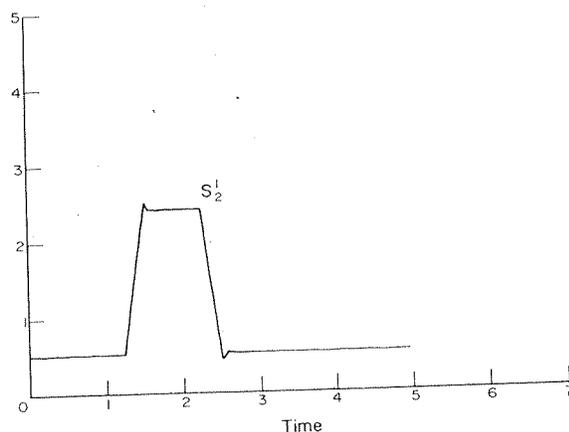


FIG. 2. Tryptophan pulse introduced into the system. Levels of various metabolites in the kynurenine pathway are computed.

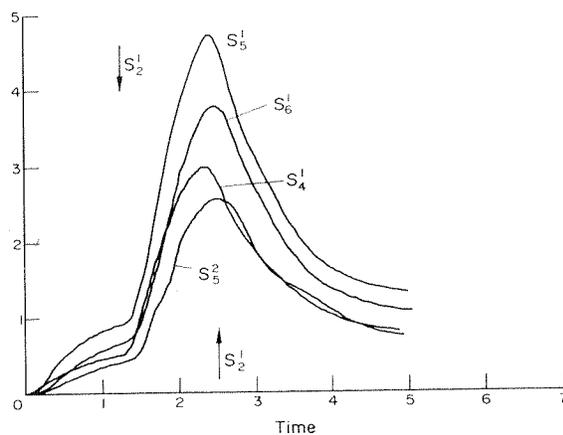


FIG. 3. Tryptophan introduced into the system by slow ramp switch. This is equivalent to the injection of a dose of tryptophan into a biological system.

E_5^1 are both activated (E_5^{*2} and E_5^{*1}), but the magnitude of activation for E_5^1 is about three times larger. It was interesting to study the coenzyme's effect under various experimental conditions. Since pyridoxal deficiency affects the kynurenine pathway metabolism, it was of interest to show in the computer simulation conditions in which the coenzyme is present and absent. The effects of B_6 concentration can be derived from those extreme values. Furthermore, in nature, because of genetic defects, enzymes E_5^1 and E_5^2 can be selectively affected and may lose their sensitivity for the coenzyme. Therefore, experiments were carried out with various conditions to demonstrate such effects.

Figures 4 and 5 show the B_6 coenzyme's effects of s_6^1 and s_5^3 , respectively. For purposes of effective demonstration, the coenzyme-reduction experiment was carried out under extreme conditions by eliminating rate constants k_{11} and k_{13} selectively. Here the tryptophan concentration was kept constant. Curve 1 in Fig. 4 shows the 3-hydroxy anthranilic acid (s_6^1) level under conditions where the coenzyme is active for both enzymes. At time zero,

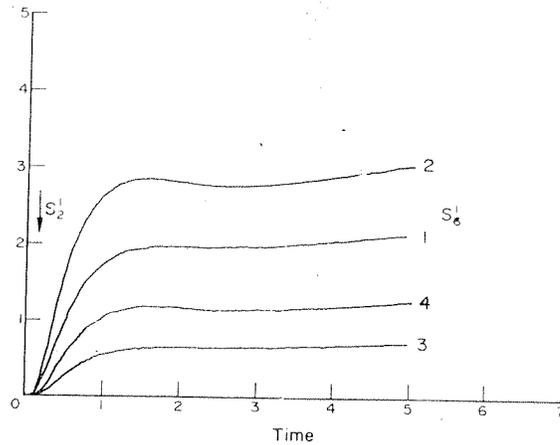


FIG. 4. The effect of coenzyme (vitamin B₆) variation on metabolite levels in the kynurenine pathway. Tryptophan is continuously maintained in the system. Enzymes E_5^1 and E_5^2 are coenzyme (C) dependent. Enzyme activity changes are simulated on the computer by coenzyme-dependent rate constants k_{11} and k_{13} . The following rate-constant combinations were used in simulation experiments during which metabolite s_6^1 was recorded:

Curve No.	Rate constants	
1	k_{11}	k_{13}
2	—	k_{13}
3	k_{11}	—
4	—	—

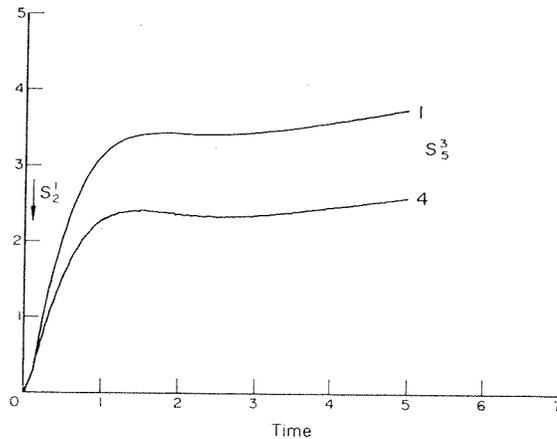


FIG. 5. Kynurenic acid (s_5^3) levels in the presence and absence of pyridoxal. Same conditions as in Fig. 4.

tryptophan is added, the concentration of s_6^1 rises rapidly, reaching a relatively steady value. There is a minor upward drift for all the curves, but this should be disregarded, since it results from the integration of electronic background noise during the long observation time. Curve 2 shows the effect when enzyme E_5^1 lacks the sensitivity for the coenzyme, with enzyme E_5^2 normal. It is evident that there is a significant rise in the s_6^1 level under these conditions. However, when the situation is reversed, with E_5^2 lacking the sensitivity

for the coenzyme, there is a drastic reduction of the s_6^1 level (curve 3). When both enzymes lose the coenzyme sensitivity or the coenzyme is absent, the s_6^1 level (curve 4) is again reduced, but to a lesser degree than in the previous case.

Since in biological systems there can be wide variations in coenzyme-sensitivity levels, it was considered worthwhile to carry out systematic experiments on coenzyme effect, on various key metabolites in the kynurenine pathway. Since this enzyme system is operationally complex, we propose only to demonstrate how each metabolite level is altered as a consequence of changes in enzyme activities. Coenzyme pyridoxal does not affect all substrate concentrations uniformly. For example, when pyridoxal is omitted from the system, substrate s_4^2 (Fig. 6) concentration is changed not only to a lesser degree than that of substrate s_6^1 (Fig. 4), but the change occurs in the opposite direction. Figure 7 shows that xanthurenic acid (s_5^2) varies over a relatively large concentration range due to the pyridoxal effect. Similar results are obtained when a tryptophan pulse is introduced (Fig. 9).

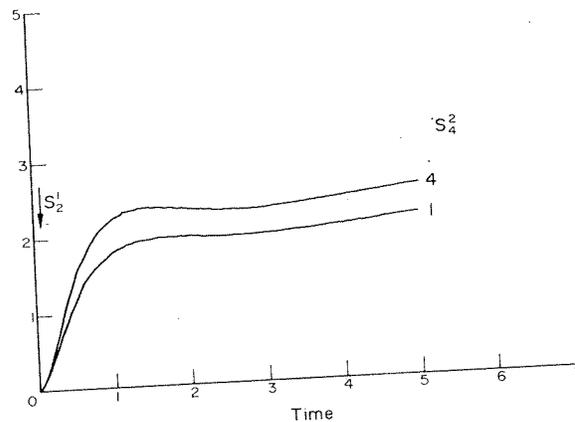


FIG. 6. N-acetyl kynurenine (s_4^2) levels in the presence and absence of pyridoxal. Same conditions as in Fig.

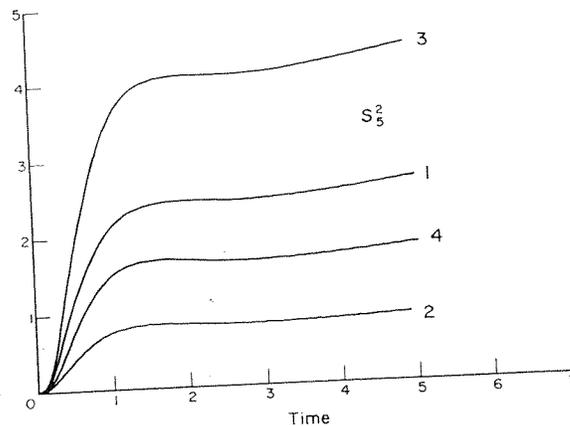


FIG. 7. Xanthurenic acid (s_5^2) levels in the presence and absence of pyridoxal. Same conditions as in Fig. 4.

FIG. 8. K

FIG. 9. Xanthu

However, in
strate concer
insignificant
key metabolit
under similar
effect pattern
pyridoxal as

While the ir
back is only su
quantitatively.

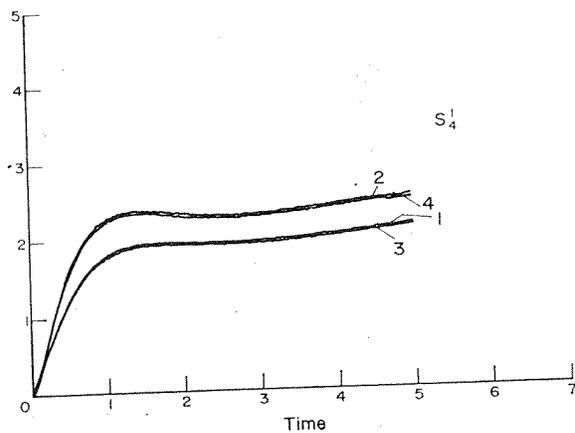


FIG. 8. Kynurenine (s_4^1) levels in the presence and absence of pyridoxal. Same conditions as in Fig. 4.

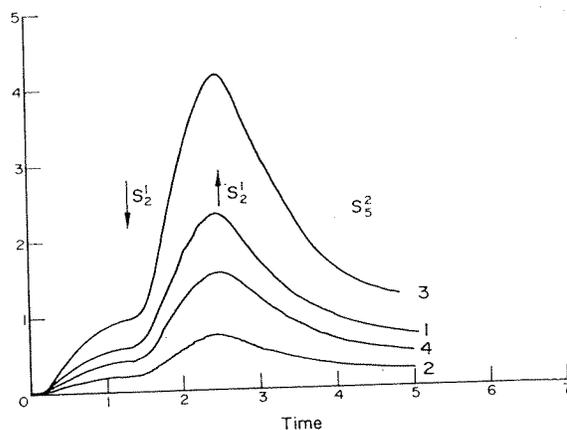


FIG. 9. Xanthurenic acid (s_5^2) levels in experiments exploring the pyridoxal effect during a tryptophan pulse. Rate constant changes are the same as in Fig. 4.

However, in the case of enzymic unbalance, the effects are opposite and variations in substrate concentrations are large. In contrast, the key metabolite kynurenine (s_4^1) shows insignificant variations due to the pyridoxal effect (Fig. 8). On the other hand, another key metabolite, 3-hydroxy kynurenine (Fig. 10) shows large variations in concentration under similar experimental conditions, using a tryptophan pulse. A different pyridoxal-effect pattern is exhibited by anthranilic acid (Fig. 11). It is highly sensitive to the effect of pyridoxal as a coenzyme, but not strongly affected by enzymic unbalance.

FEEDBACK INHIBITION

While the inhibition of the enzyme tryptophan pyrolase (E_2^1) by kynurenine (s_4^1) feedback is only suggested, it is, nevertheless, considered worthwhile to explore this phenomenon quantitatively. In order to demonstrate this effect, the s_4^1 level was built up by reducing

is in Fig. 4.

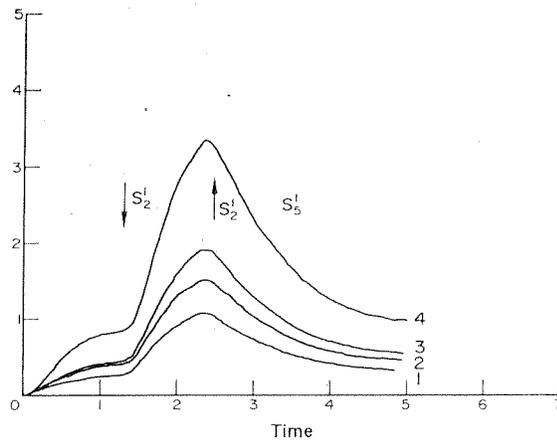


FIG. 10. 3-Hydroxykynurenine (s_5^1) levels during tryptophan pulse. Same conditions as in Fig. 4.

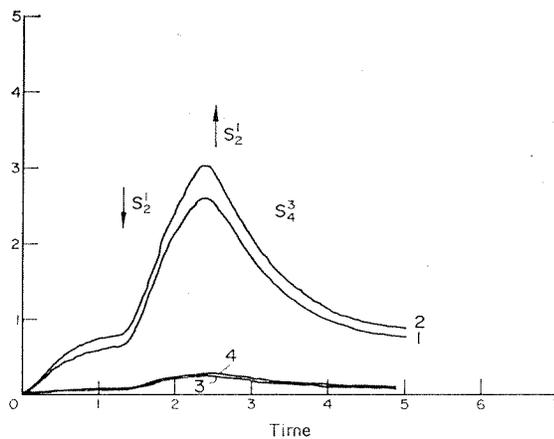


FIG. 11. Anthranilic acid (s_4^3) levels during tryptophan pulse. Same conditions as in Fig. 4.

the rate constant k_9 , which principally controls the s_4^1 turnover. Figures 12 and 13 show the effects of s_4^1 inhibition on various metabolites. It is indeed evident that feedback inhibition effectively controls the level of kynurenine when k_9 is varied over a wide range (0.33–1.0). Significant changes occur in the s_6^1 and s_5^1 concentrations, but extreme changes occur in the s_5^2 levels. It is likely that in actuality this system contains some other regulatory features to maintain stability of metabolite levels.

DISCUSSION

The model system was based on the currently established tryptophan–kynurenine pathway in the liver. This information is greatly deficient since it does not contain data on any regulatory features with respect to enzyme action as well as enzyme synthesis. It is well known that the synthesis of the first input enzyme, tryptophan pyrolyase, is controlled

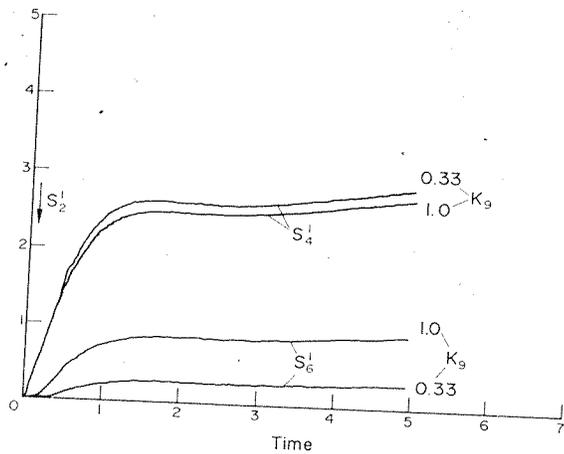


FIG. 12. Inhibitory effect of kynurenine (s_4^1) on kynurenine hydroxylase (E_4^1). Kynurenine and 3-hydroxy-anthranilic acid levels in the presence and absence of inhibition. Tryptophan present continuously in the system. The inhibitory process was simulated on the computer by altering the value of rate constant k_9 . Normal enzyme activity ($k_9=1.0$) and inhibited activity ($k_9=.33$).

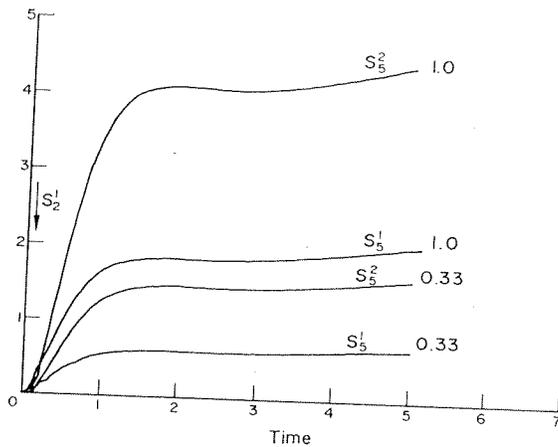


FIG. 13. Hydroxykynurenine (s_5^1) and xanthurenic acid (s_5^2) levels. Experimental conditions as in Fig. 12.

by hormones as well as by tryptophan itself. However, an enzyme system as complex as this certainly contains many other regulatory features.

Extensive clinical measurements of the intermediates of tryptophan metabolism in urine are carried out with the objective of relating various diseases with the urinary-metabolic pattern. There is no way to compare the computer-model analysis with this clinically observed data. The computer model shows only the flow of metabolites within an enzyme system. It does not take into account how these metabolites are distributed in tissue, blood and urine. Usually it is assumed that there is a certain proportionality between liver and urinary metabolites. This does not have to be so, but for a rough measure, such an assumption could be made.

Our model-system analysis was carried out with a rather negative objective, namely to show that sampling and measurement of a few metabolites in a complex metabolic system will not yield adequate information about the system nor permit us to deduce any mechanisms

which could cause abnormal distribution of metabolites in urine. It has to be noted that the pyridoxal-sensitive enzymes, kynureninase (E_5^1) and kynurenine transaminase (E_5^2), which primarily control the distribution of metabolites, definitely affect the metabolite-distribution pattern. This results from the balance between these two enzymes with respect to their concentrations as well as their coenzyme sensitivity. While genetic factors can influence the level of an enzyme as well as its coenzyme sensitivity, one may assume that such factors may be contributory to symptoms of certain types of disease. More significantly, synthesis of these enzymes may be regulated independently by mechanisms which are currently unknown. In conditions of disease where hormonal and metabolite unbalances may appear, unbalance, as a consequence, could appear in the enzymic-activity pattern.

Our computer-simulation studies indicate that unbalanced enzymic activity can produce a variety of metabolite patterns. They suggest that, unless the underlying features of the metabolic control of enzymic activity as well as enzyme levels are understood, metabolite patterns *per se* do not yield significant information. The attempt is made here to analyze computer-simulation experiments. These merely show the possibility of pitfalls in interpreting clinical metabolite patterns. A profitable approach to the problem can only be made when the regulatory features of tryptophan metabolism have been established experimentally. This would enable us to devise a more realistic model system. Then quantitative analysis of enzymic activities could be performed under a variety of conditions, and it would be possible also to analyze the relations of various metabolic patterns under the conditions of various diseases. The current clinical-measurement approach is unproductive and has rather limited significance.

Acknowledgement—We are highly indebted to JAMES O. BROOKS for carrying out the analog computer programming and operating the computer in a highly competent manner at AVCO Analog Computer Center, Wilmington, Mass.

REFERENCES

1. E. C. AZMITA and B. S. MCEWEN, Corticosterone regulation of tryptophan hydroxylase in midbrain of the rat, *Science* **166**, 1274 (1969).
2. W. E. KNOX and M. M. PIRAS, Tryptophan pyrrolase of liver. III. Conjugation *in vivo* during cofactor induction by tryptophan analogues, *J. Biol. Chem.* **242**, 2959 (1967).
3. M. M. PIRAS and W. E. KNOX, Tryptophan pyrrolase of liver. II. The activating reactions in crude preparations from rat liver, *ibid* **242**, 2952 (1967).
4. F. HEINMETS, Model systems for hormonal induction and regulation of enzyme synthesis, *Physiol. Chem. Phys.* **2**, 351 (1970).
5. F. HEINMETS, Hormonal induction of enzymes by computer simulation and model system analysis, *Physiol. Chem. Phys.* **3**, 47 (1971).
6. F. HEINMETS, Computer simulation and analysis of a model system for tryptophan metabolism in the pineal gland, *Physiol. Chem. Phys.* (in press).
7. L. MUSAJO and C. A. BENASSI, Aspects of disorders of the kynurenine pathway of tryptophan metabolism in man, *Adv. Clin. Chem.* **7**, 63 (1964).
8. J. M. PRICE, R. B. BROWN and N. YESS, Testing the functional capacity of the tryptophan-niacin pathway in man by analysis of urinary metabolites, *Adv. Metab. Dis.* **2**, 149 (1965).
9. K. ALTMAN and O. GREENGARD, Correlation of kynurenine excretion with liver tryptophan pyrrolase levels in disease and after hydrocortisone induction, *J. Clin. Invest.* **45**, 1527 (1966).
10. H. SPIERA, Excretion of tryptophan metabolites in rheumatoid arthritis, *Ann. N.Y. Acad. Sci.* **166**, 61 (1969).
11. W. W. COON and E. NAGLER, The tryptophan load as a test for pyridoxine deficiency in hospitalized patients, *ibid* **166**, 30 (1969).
12. R. R. BROWN, D. P. ROSE, J. M. PRICE and H. WOLF, Tryptophan metabolism as affected by anovulatory agents, *ibid* **166**, 44 (1969).
13. C. WAGNER, Regulation of the tryptophan-nicotinic acid-DPN pathway in the rat, *Biochem. Biophys. Res. Commun.* **17**, 668 (1964).
14. F. HEINMETS, *Quantitative Cellular Biology*. Marcel Dekker, New York (1970).