

AMINO ACID DIGESTIBILITY IN NON-RUMINANTS - A REVIEW

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SUMMARY

Approaches to the determination of dietary amino acid digestibility in monogastric farm animals are reviewed. In the growing pig, it appears that measurement of digestibility at the end of the ileum is justified but the situation is not so clear for poultry. Regardless of the species, the amino acid flow at the end of the ileum should be corrected for its endogenous component to give estimates of true digestibility. Routine approaches to determining amino acid digestibility are discussed and distinction between the measures of amino acid digestibility and availability is stressed.

INTRODUCTION

The digestibility of amino acids in feeds is highly variable. By way of example, amino acids in a protein source such as casein are almost completely released during digestion whereas for a feedstuff such as meat-and-bone meal more than half the amino acids may remain unabsorbed from the animal's digestive tract. Accurate data on the digestibility of amino acids in feeds is needed, therefore, to allow the animal's daily requirement for individual dietary amino acids to be met more precisely and economically. The amino acid requirement should be defined as that amount needed to maximise profitability for the particular production unit in the short- to medium-term. Requirement values themselves should not be viewed as static, therefore, but rather they vary both spatially and temporally. A dynamic approach to estimating amino acid requirements is now afforded by computerised models simulating animal growth (Moughan and Verstegen 1988).

The aim of the present contribution is to briefly review the currently-used in vivo methods for determining amino acid digestibility in non-ruminants and to assess their adequacy. In the future, it is likely that poorly-digestible feedstuffs will be used increasingly in animal production and there will be an even greater need than at present for practical yet reliable digestibility assays.

PROTEIN DIGESTION

After being ingested by the animal, dietary protein becomes progressively mixed with endogenous proteins and the total is subjected to digestive breakdown in the upper alimentary tract. Free amino acids or small peptides, released by the digestive enzymes, are absorbed anterior to the end of the small intestine. At the terminal ileum there will be an amount of protein which has remained undigested and peptides and free amino acids which have not been absorbed. These along with other undigested dietary components will pass into the large intestine whereby they are subject to the action of a dense population of microorganisms. Some protein, peptides and free amino acids may escape breakdown in the hindgut and be excreted in the faeces, but a considerable proportion of the nitrogenous material entering the hindgut will be metabolised by the microflora. Also, non-protein nitrogen (mainly urea) may enter the hindgut from the animal's bloodstream and be used for the microbial synthesis of amino acids and microbial protein. The hindgut microbes are capable of intense proteolytic activity, with the concomitant release of free amino acids. It appears, however, that amino acids are not absorbed across the large intestinal mucosa to any significant extent (Wrong et al. 1981).

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The microbial metabolism of nitrogenous compounds has been the subject of several comprehensive reviews (Rerat 1981; Mason 1984; McNeil 1988; Low and Zebrowska 1989). At least for the growing pig and for most amino acids in most feedstuffs there is a net loss of amino acid between the terminal ileum and the rectum. The amino nitrogen is absorbed from the hindgut mainly as ammonia, which under normal circumstances is of no nutritional value to the host. Especially for methionine and sometimes lysine there may be a net synthesis of the amino acid due to the microbial action. An indication of the significance of the hindgut microflora metabolism is that around 80% of faecal nitrogen is present in microbial bodies (Low and Zebrowska 1989). The important implication of this, in practice, is that only a very low proportion of the faecal amino acid excretion is directly related to the flow of undigested dietary amino acids entering the large intestine.

The above brief and simplified summary of protein digestion should serve to highlight the inadequacies of the traditional faecal measures of amino acid digestibility. Because of the microbial action in the hindgut, and that at least for most species of animal amino acids from the hindgut do not become available for body protein synthesis, faecal digestibility coefficients are likely to be misleading. Measurement of amino acid flow and digestibility at the end of the ileum (Payne *et al.* 1968) is now generally recognized as a more acceptable approach, at least theoretically (Rerat 1981; Tanksley and Knabe 1984; Sauer and Ozimek 1986).

Although, the effect of hindgut microbial metabolism on protein digestion does appear to be a rather general phenomenon (Table 1), it is not necessarily of practical significance in all cases. The extent of microbial activity and thus the degree of difference between ileal and faecal digestibility coefficients depends on the type and numbers of microorganisms present, the type of feedstuff and the time of residence of material in the hindgut. It is thus a function of both species of animal and diet. The practical importance of differences in amino acid digestibility as determined using the ileal or faecal methods will now be addressed with reference to the commercially-important monogastric species, pigs and chickens. To allow comparison between the different types of digestibility measurements, however, requires that methods be developed to allow adequate collection of ileal digesta, and this in itself has not been straightforward.

DIGESTA COLLECTION WITH PIGS

Numerous methods have been developed to allow the total collection of digesta or sampling of digesta from the terminal ileum of pigs. In the main, these methods involve the surgical implantation of cannulae. The different approaches to cannulation have been the subject of recent reviews (Sauer *et al.* 1989a; Low 1990) and it is concluded that more work is required before firm conclusions can be drawn as to the superiority of any one procedure. At this stage, however, some general comments can be made. Ileo-ileo and ileo-caecal re-entrant cannulation involve total transection of the ileum and this is considered to be undesirable. The ileo-colic (post-valve) re-entrant cannulation, post-valvular T-caecum cannulation and simple T-ileum cannulation all have the distinct advantage that the function of the ileo-caecal valve is preserved and the ileum is not transected. When simple T-cannulation of the ileum is adopted the surgery is less invasive than with the other two approaches but because digesta are sampled there is reliance on an indigestible marker compound. The post-valve T-caecum method has the advantage that during collection most of the digesta pass through the cannula because the ileo-caecal valve protrudes directly into the cannula. Indeed, the post-valve T-caecum technique (van Leeuwen *et al.* 1988) would appear to be the current method-of-choice, but its superiority over the simple T-cannulation of the ileum has yet to be demonstrated.

TABLE 1 Comparison of the ileal and faecal digestibility of dietary protein for the chicken and several simple-stomached mammals

Apparent digestibility		
	Ileal	Faecal
Piglet ^a	0.90	0.97
Growing Pig ^b	0.66	0.81
Pre-ruminant calf ^c	0.88	0.94
Adult Human ^d	0.87	0.89
Chicken ^e	0.78	0.86
Growing rat ^f	0.69	0.78
^a	Six kg liveweight piglets fed bovine milk (Moughan <i>et al.</i> 1990a)	
^b	Forty five kg liveweight pig given meat and bone meal based diet (Moughan <i>et al.</i> 1984)	
^c	Milk-fed calf (45 kg liveweight), (Moughan <i>et al.</i> 1989a)	
^d	Sixty-five kg adult human consuming a meat, vegetable, cereal, dairy product diet (Moughan and Rowan 1989)	
^e	Overall mean amino acid digestibility for 9 amino acids and 16 diets given to 10-week-old chickens (Raharjo and Farrell 1984)	
^f	Eighty g liveweight rat given a meat and bone meal based diet (Moughan <i>et al.</i> 1984)	

The potential impact of any form of cannulation on the normal physiological functioning of the animal, however, should not be overlooked. Livingstone and McWilliam (1985) reported that pigs with simple T-cannulae implanted in the ileum had similar voluntary feed intakes to their non-cannulated sisters but grew more slowly and less efficiently. Wenham and Wyburn (1980) in radiological studies with sheep found that several types of cannulation, including simple T-piece cannulation, caused some disruption to normal digesta flow.

An alternative to collecting digesta via intestinal cannulae, is to sample digesta from the terminal ileum of animals while under anaesthesia (Moughan *et al.* 1989b). The so-called slaughter technique, has the distinct advantage of involving minimal disruption of normal digestive function in the animal and allows samples of digesta to be taken from several parts of the digestive tract. The main technical criticism of this method concerns the potential difficulty of obtaining representative samples of digesta. However, and based on the experience of the authors, when a frequent feeding regime is adopted in combination with the slaughter technique, digestibility data are no more variable than those found with cannulated animals.

All of the above-described methods of digesta collection in the pig are expensive and somewhat laborious. A simpler technique, which has been widely used in practice, is ileo-rectal anastomosis. However, and although the method has a number of logistical advantages, there are still serious doubts concerning the physiological normality of anastomised animals (Moughan 1991a). A comparison of the different methodologies available including detailed study of their potential effects on the pig's digestive physiology, is long over-due. In the

meantime, it appears that post-valvular cannulation of the caecum using a simple T-piece cannula or the slaughter method are likely to yield the most reliable results.

DIGESTA COLLECTION WITH CHICKENS

Given the small size of the chicken relative to the pig, it has been common to collect ileal digesta using the slaughter method (Payne *et al.* 1968; Varnish and Carpenter 1975; Achinewhu and Hewitt 1979; Raharjo and Farrell 1984), but simple T-piece cannulation has also been employed (Raharjo and Farrell 1984; Summers *et al.* 1982; Gurnsey *et al.* 1985; Crissey and Thomas 1987). Thomas and Crissey (1983) commented on the considerable difficulties encountered in practice due to loss of cannulae from the body. Polypropylene cannulae would appear to offer some advantages in the latter respect. With the slaughter method, there are a number of technical aspects that need to be considered, such as feeding method, method of killing, means of removing digesta, length of ileum sampled and the indigestible marker employed. To date there has been little experimental work to evaluate these factors. Summers and Robblee (1985) reported no differences in the ileal digestibility of dietary amino acids between anaesthetised and killed broiler chickens. At our own Centre (Y. Kee Hor, R. King and P.J. Moughan, unpublished data) euthanasia of birds using the barbiturate sodium pentobarbitone has been found superior to carbon dioxide asphyxiation and the work of Bolton (1964) would indicate that death by cervical dislocation may cause agonial spasms with an accompanying movement of digesta between different parts of the tract. With respect to removal of digesta from the tract, some workers have used manual manipulation with apparently satisfactory results (J. van der Klis, pers. comm.). This procedure may lead to a shedding of mucosal cells, however, and our group has preferred collecting digesta by gentle flushing of the ileal contents with distilled water. No effect on dietary nitrogen digestibility was found consequent upon flushing with distilled water or physiological saline (Y. Kee Hor, R. King and P.J. Moughan, unpublished data). Chromic oxide has been the most frequently used indigestible marker compound but there has been no definitive study to validate its use in the broiler ileal assay. Work is urgently required to assess the effects of factors which may impact upon the assay, to allow specification of a standardised procedure. Until this is achieved results generated by ileal assays are open to interpretation and any comparison with other assay procedures is hampered. Also, and although cannulation procedures have been employed there has been no thorough evaluation of the possible effects of cannula implantation on digestive physiology in the fowl.

Rather than collect ileal digesta from the chick by either cannulation or following slaughter, which is costly and poses a number of difficulties, some workers have tried to avoid the influence of the hindgut microflora by collecting excreta (faeces and urine combined), but after caecectomy. Microbial activity in the hindgut is reduced with caecectomy (Low 1990) but it may not be eliminated (Whitacre and Tanner 1989). The effects of removing the caecae on digestive physiology are unknown and the fact that urine which contains non-amino nitrogen and amino acids is voided in the excreta, to some extent confuses the interpretation of the "availability" data.

ILEAL VERSUS FAECAL DIGESTIBILITY - PIGS

Numerous studies, employing a variety of methods for collecting ileal digesta, have been reported whereby the ileal and faecal digestibilities of dietary amino acids in pigs have been determined. There is general agreement that the ileal digestibilities of most amino acids are lower than corresponding digestibilities determined over the entire digestive tract (Table 2). According to Zebrowska (1978) the amount of amino acids disappearing in the large intestine

usually varies from 5 to 35% of the total amino acids ingested. It appears that the lower the ileal digestibilities of nitrogen and amino acids, the greater is the difference between ileal and faecal digestibilities (Table 3). This is understandable, as with diets containing highly digestible protein most is absorbed before the digesta enter the large intestine whereas with protein sources of lower quality there are larger residues to allow a disappearance of amino acids between the terminal ileum and rectum.

TABLE 2 Ileal and faecal digestibilities of essential amino acids in pig diets^a

Amino acid	Location		
	Ileum	Faeces	Difference
Arginine	0.88	0.92	0.04
Histidine	0.85	0.92	0.07
Isoleucine	0.81	0.87	0.06
Leucine	0.83	0.89	0.06
Lysine	0.85	0.87	0.02
Methionine	0.85	0.85	0.00
Phenylalanine	0.82	0.89	0.07
Threonine	0.73	0.85	0.12
Tryptophan	0.79	0.89	0.10
Valine	0.79	0.87	0.08
Average	0.82	0.88	0.06

^a From Sauer and Just (1979), n = 30

TABLE 3 Apparent digestibilities of some amino acids in wheat flour and wheat offal measured at the terminal ileum (I) and in faeces (F)^a

Amino acid	Wheat flour		Wheat offal	
	I	F	I	F
Lysine	0.84	0.86	0.66	0.76
Histidine	0.91	0.94	0.79	0.88
Methionine	0.94	0.94	0.78	0.82
Isoleucine	0.94	0.95	0.73	0.75
Leucine	0.95	0.96	0.75	0.79

^a From Sauer *et al.* (1977)

In the pig, it is generally agreed that the amount of amino acid absorbed in the small intestine up to the terminal ileum gives a more reliable estimate of the amount available to the animal than does the conventional faecal index method, particularly if the diet contains protein of low quality. There is, however, a need for some caution in the interpretation of ileal digestibility values because of microbial fermentation that occurs in the upper digestive tract (Cranwell 1968; Bergner *et al.* 1986; Dierick *et al.* 1986a,b). Further, ileal digesta contain endogenous proteins which confuses the interpretation of apparent digestibility coefficients. Nevertheless, apparent ileal digestibility coefficients have been shown to be sensitive in detecting small differences in protein digestibility due to the processing of foods (Rudolph *et al.* 1983; Vandergrift *et al.* 1983; van Weerden *et al.* 1985; Sauer and Ozimek 1986; Knabe

et al. 1989). Also, several studies (Tanksley and Knabe 1980; Low *et al.* 1982; Just *et al.* 1985; Moughan and Smith 1985; Laplace *et al.* 1985; Dierick *et al.* 1988; Laplace *et al.* 1989) have demonstrated that apparent ileal digestibility coefficients accurately describe the extent of amino acid uptake from the gut, at least for a range of commonly used feedstuffs which have not been subjected to high temperatures during their processing. Amino acid digestibilities can be predicted more precisely from ileal nitrogen digestibility than from faecal nitrogen digestibility. However, neither ileal nor faecal nitrogen could be used with a high degree of certainty to predict ileal amino acid digestibilities (Knabe *et al.* 1989).

Several attempts have been made to collate data from the wider literature on ileal amino acid digestibility in the pig, but care must be taken when comparing data for different ingredients, generated using different methods. The data on ileal amino acid digestibility for the growing pig, presented by Rhone Poulenc Nutrition (1989), are particularly useful in that a single method (ileo-rectal anastomosis) was used to generate information on a wide range of feedstuffs.

ILEAL VERSUS EXCRETA DIGESTIBILITY - CHICKENS

The influence of the hindgut microflora in chickens on amino acid digestibility is not as clearly established as for the pig. The chicken has a relatively small hindgut and food moves rapidly through the digestive tract. The role of the hindgut microflora in fowl has been the subject of recent review (Austic 1983; Thomas and Crissey 1983; van Weerden 1989; Whitacre and Tanner 1989; Johnson 1990) and there is a growing consensus that there may be significant microbial fermentation in the lower gut of the chicken and that this should be accounted for in digestibility assays. However, there is not complete agreement. Papadopoulos (1985), after reviewing the subject for example, concluded that ileal and faecal assays will lead to similar results. There is universal agreement, however, that more comparative studies are required to fully resolve this debate.

As for the growing pig, an effect of the hindgut microflora is likely to be greatest for feedstuffs of low digestibility. This is evidenced by the data from a study by Johns *et al.* (1986), albeit using caecectomised cockerels, of the true digestibility of amino acids in a heat-treated meat and bone meal (Table 4). There were differences between the intact and caecectomised birds for the basal meat and bone meal with the differences being magnified after heat treatment of the meal. Such a difference in digestibility may even be higher if measurement was made at the terminal ileum.

Overall it seems that there is a significant degree of microbial activity in the hindgut of the chicken and there would appear to be a case for developing a standard ileal digestibility assay. An ileal assay has the added advantage of not being affected by urinary amino acid excretion which may confound digestibility measures based on excreta. However, it appears (Low and Zebrowska 1989; Moreto and Planas 1989) that amino acids may be absorbed from the hindgut of birds, which does not appear to be the case for mammals. If this is true and occurs to a significant extent, then the ileal digestibility assay may not be valid. If, on the other hand, it can be demonstrated that there is negligible absorption of intact amino acids and peptides by the colonic and caecal mucosa, then standardised ileal digestibility assays for broiler and layer birds would seem to offer significant advantages to the poultry industry. Work in this area with poultry is not as advanced as with the pig. The potential for hindgut amino acid absorption in birds needs to be examined. Standardised ileal assays need to be developed and tested. A comprehensive ileal/excreta (faecal) comparison

TABLE 4 True digestibility of amino acids in diets containing heat-treated meat and bone meals determined using intact (I) and caecectomised (C) cockerels

Amino acid	Diet ^a	
	1	2
Lysine		
I	0.88	0.58
C	0.82	0.45
Methionine		
I	0.90	0.79
C	0.91	0.76
Threonine		
I	0.79	0.62
C	0.75	0.55

^a Diet 1 contained (10%) meat and bone meal ex the processing factory.
Diet 2 contained (10%) meat and bone meal from the same batch as used in Diet 1, but after 5 hours heating in a steam-jacketed cooker.

needs to be made and finally ileal amino acid digestibility data should be evaluated for their usefulness in practical dietary formulation.

TRUE AND APPARENT DIGESTIBILITY

Accepting that amino acid digestibility should be based on measurements made at the terminal ileum of mammals and birds, it needs to be recognised that ileal digesta contain appreciable quantities of non-dietary protein from sources such as bacteria, hair, digestive secretions, mucus and cells. To get a proper or "true" estimate of digestibility, correction should be made for the non-dietary component. True digestibility estimates should more closely describe the uptake of amino acids from the digestive tract. True digestibility has the advantage over apparent digestibility in that it is a fundamental property of the feedstuff, being independent of dietary conditions. For a given amino acid, the apparent digestibility increases exponentially with the ingested quantity because endogenous excretion, as a percent of total excretion, decreases proportionally. By contrast, true amino acid digestibility is not affected by the ingested quantity. Therefore using true digestibility data allows raw materials to be accurately compared, even if they are ingested in different quantities. The benefits of using true as opposed to apparent digestibility coefficients is discussed more fully elsewhere in the present proceedings (Moughan 1991b).

Although the need for correction of apparent amino acid digestibility values for endogenous excretions is recognised, there are problems in attempting to apportion amino acids appearing in ileal digesta to dietary or endogenous origin. In the past two approaches have been adopted to quantify endogenous levels of amino acids appearing at the terminal ileum. These are analysis of ileal digesta from animals given a protein-free diet and the feeding of graded amounts of a single protein source followed by extrapolation to zero intake of amino acids of the linear regression of ileal amino acid output on dietary amino acid intake. Both methods are, however, open to criticism. Further, there is evidence that

with both methods, the level and source of dietary fibre influences the outcome (Holmes *et al.* 1974; Sauer 1976; Taverner *et al.* 1981), probably through an effect on mucin production (Taverner 1979). Again, natural fibre may behave differently from cellulose, frequently used as a fibre source in experimental semi-purified diets.

A practical method for determining endogenous ileal amino acid flow which is not subject to the criticisms of the protein-free or regression methods but which is applicable only to protein sources which do not contain fibre or antinutritional factors (e.g. meat and bone meal, fish meal, dried yeast, blood meal, milk powder) has been recently proposed (Moughan *et al.* 1990b) and evaluated. Although the latter technique appears to be useful for this restricted group of feeds, at present there is no satisfactory practical approach for determining endogenous loss in the remaining feedstuffs used in pig and poultry production.

CONCLUSION

True ileal amino acid digestibility appears to be the method of choice for determining dietary amino acid absorption from the gastrointestinal tract of the pig and is probably also a useful method with poultry. A drawback, particularly with pig ileal assays, is their cost. Development of a routine relatively inexpensive ileal assay would have appeal. In this respect the laboratory rat offers much promise. Digestive physiology is similar between rats and pigs, so it is not surprising that when apparent ileal amino acid digestibility has been compared between the species (Moughan *et al.* 1987; Donkoh *et al.* 1990; Smith *et al.* 1990) close agreement has been observed (see Table 5).

TABLE 5 Mean apparent ileal amino acid digestibilities in ground barley, meat and bone meal and a compound diet, determined in three separate studies using the laboratory rat and growing pig

Amino acid	Barley ^a		Meat and bone meal ^b		Compound diet ^c	
	Rat	Pig	Rat	Pig	Rat	Pig
Lysine	0.75	0.78	0.81	0.82	0.77	0.76
Methionine	0.75	0.84***	0.83	0.84	-	-
Threonine	0.68	0.72	0.73	0.73	0.71	0.72
Histidine	0.76	0.78	0.74	0.75	0.77	0.78
Isoleucine	0.80	0.78	0.80	0.81	0.76	0.76
Leucine	0.78	0.80	0.78	0.78	0.80	0.77
Phenylalanine	0.76	0.82*	0.81	0.83	0.80	0.76
Valine	0.79	0.80	0.76	0.80	0.76	0.75

^a Moughan *et al.* (1987)

^b Donkoh *et al.* (1990)

^c Smith *et al.* (1990)

* Significant $P < 0.05$, *** Significant $P < 0.001$

In these studies, ileal digesta were collected from the euthanased animal and considerable preliminary work was undertaken to define optimal sampling conditions, to ensure a valid inter-species comparison. Work is continuing at our Centre to further validate the rat model. When validity has been established for a particular feedstuff, and it should be stressed that the rat may not be a useful model in all cases, the rat assay can be used to generate data quickly and relatively inexpensively.

A further simple and rapid method which may have potential for the determination of ileal amino acid digestibility at least in pigs, is the nylon bag technique (Sauer et al. 1989b). This method warrants further investigation and may have value as a rapid screening test. The *in vitro* approach can also provide digestibility data rapidly, cheaply and with impressive precision. It is very difficult, however, to adequately simulate the complex processes occurring during digestion *in vivo*, and *in vitro* assays generally have not provided accurate estimates of amino acid or nitrogen digestibility.

It is important when discussing protein digestion to distinguish between the concepts of digestibility and availability. Digestibility refers to the uptake of an amino acid from the gut whereas availability refers to the degree of uptake and subsequent utilization of the amino acid for protein synthesis and other anabolic processes. Amino acid availability is a complex phenomenon affected by many interacting factors (Moughan 1991a). There is likely to be a discrepancy between digestibility and availability, particularly for the amino acid lysine because of its free E-amino group, for heat-treated foods (Moughan 1989). On the one hand, chemically unavailable lysine may be absorbed and then not utilized but seemingly more importantly, lysine digestibility coefficients themselves are likely to be inaccurate, at least for some processed foods. With the early stages of the Maillard reaction, for example, which are predominant under the normal conditions of food processing, the deoxyketosyl lysine derivative (Amadori compound) formed is hydrolysed back to lysine in the presence of strong acids. Thus conventional amino acid analysis leads to overprediction of the actual lysine present in food or ileal digesta from an animal fed the processed feedstuff. Consequently, the ileal lysine digestibility coefficients are likely to be biased, and to an unknown degree. Also, and for feedstuffs generally, it is to be expected as noted by Batterham *et al.* (1990a) that digestibility values will overestimate availability. A proportion of the absorbed amino acids, including the first-limiting amino acid will be inevitably catabolised by the animal with the degree of such catabolism varying with the level of uptake (Moughan 1991b). For this same reason, absolute values for body lysine retention can not be used to assess the adequacy of ileal digestibility coefficients (Batterham *et al.* 1990b).

In conclusion, true ileal amino acid digestibility coefficients are likely to be useful indicators of amino acid absorption for feedstuffs in which the constituent amino acids have not undergone structural changes during processing or storage. In feedstuffs where chemically unavailable amino acids are present in significant quantities, however, ileal digestibility coefficients should not be expected to accurately indicate amino acid absorption, at least for some of the amino acids. Finally, digestibility assays should be evaluated in terms of their accuracy for predicting the overall level of absorption of an amino acid from the digestive tract. As such they indicate the amount of a dietary amino acid potentially available for metabolism and thus have a role in the practice of diet formulation, but they do not indicate the extent to which an amino acid will actually be used for protein synthesis. The latter depends upon the interaction of several dietary and animal factors. The reason why truly absorbed chemically-available amino acids may not be used for body protein accretion is in itself a topic worthy of more detailed investigation.

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