elastoidin, which has a high shrinkage temperature of about 64°, but a low hydroxyproline content similar to that of fish collagen. The reversibility of thermal shrinkage shown by elastoidin in its natural state also indicates the possibility of the presence in elastoidin of cross-linkages that do not occur in other collagens, since these manifest reversible shrinkage only after treatment with formalin (Gerngross & Katz, 1926). The cross-linking may also afford an explanation for the slowness with which elastoidin is converted into gelatin even under drastic treatment with steam under pressure.

SUMMARY

1. The amino acid composition of elastoidin from the ceratotrichia of *Carcharinus melanopterus* has been determined.

2. The amino acid distribution follows the general pattern characteristic of collagens, and justifies the inclusion of elastoidin in the collagen group of proteins made on the basis of physical studies.

3. Elastoidin differs from other collagens, being rich in tyrosine and containing a significant amount of cystine. These differences may explain the peculiar hydrothermal behaviour of elastoidin which distinguishes it from other collagens.

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Comparison of the Metabolism of Uniformly ¹⁴C-labelled L-Phenylalanine, L-Tyrosine and L-Tryptophan in the Rat

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In the adult animal whose metabolism is in the steady state the amount of ingested tryptophan incorporated into body protein should be balanced by liberation of an equivalent amount of endogenous tryptophan by protein breakdown. The observed degradation products of tryptophan should therefore account for an amount of tryptophan equal to that ingested. In fact, as is shown in the subsequent discussion, this is very far from being the case. Our knowledge of the metabolism of

tryptophan differs from that of the metabolism of phenylalanine and tyrosine in a most important respect. Phenylalanine is metabolized via tyrosine to the metabolically highly reactive acetoacetic and fumaric acids, so that metabolism of large amounts of phenylalanine and tyrosine can be readily accounted for. But there is no pathway at present known for conversion of the aromatic ring of tryptophan into metabolically reactive aliphatic compounds.

It was hoped that light might be thrown on this problem by comparing the metabolism of uniformly ^{14}C -labelled L-phenylalanine, L-tyrosine and L-tryptophan in the rat. The results described in this paper show that a fairly readily available pathway (or pathways) for degradation of tryptophan to aliphatic substances must exist, and suggestions are made concerning its nature.

EXPERIMENTAL

Animals. Male white rats weighing between 190 and 210 g. were taken from the School stock as required. A total of eighteen animals was used. They were starved overnight (16 hr.) before administering the amino acid in solution by stomach tube.

Amino acids. Uniformly ^{14}C -labelled L-tyrosine, L-phenylalanine and L-tryptophan were prepared biosynthetically in one of two ways (C. E. Dalgliesh & R. W. Dutton, paper in preparation). In the first procedure uniformly labelled carbohydrate prepared by photosynthesis with tobacco leaves was used as substrate for growing *Torulopsis utilis*. The yeast protein was isolated and hydrolysed enzymically, and the amino acids were separated by column chromatography. In the second procedure direct photosynthesis by *Chlorella vulgaris* was used. The uniformity of labelling was inferred from the biosynthetic procedures, and was not confirmed by degradation. The amino acids used had the following activities: L-tyrosine 7 or $10\text{ }\mu\text{C}/\text{mg.}$; L-phenylalanine 7 or $10\text{ }\mu\text{C}/\text{mg.}$; L-tryptophan 10 or $2\text{ }\mu\text{C}/\text{mg.}$

Experimental procedure. Immediately after administration of the labelled amino acid the animal was placed, without food or water, in a metabolism apparatus similar to that devised by Lifson & Lorber (1954), allowing collection of respiratory CO_2 , urine and faeces. After the appropriate time the animal was removed from the apparatus, anaesthetized with ether and rapidly dissected, and as much blood as possible removed from the aorta with a heparinized syringe. The liver, kidney, spleen, gut (including pancreas), heart, lung and brain were rapidly removed into a freezing mixture. One hind leg was then removed and the skin, muscle and bone were separated. It was assumed that the skin, muscle and bone of a hind leg were typical of the corresponding tissues of the whole animal, and calculations were based on the assumption that total muscle represented 50% of the body weight and that the weight of bone in the whole body was seven times that in the dissected leg. (These values were determined in a preliminary experiment.) In none of our experiments was significant activity found in the skin.

The treatment of the individual organs is exemplified by the following procedure for liver. The liver was washed, and weighed after removal of excess of water. A portion was then removed, weighed, cut up and dried in an oven at $100\text{--}120^\circ$ for 12 hr. The dry residue was powdered and used for determination of total activity. The remaining portion of the liver was homogenized with 30 ml. of distilled water. The homogenate was placed for 5 min. in a boiling-water bath to coagulate the bulk of the protein. Trichloroacetic acid solution (30%, w/v) was added to a final concentration of 5% (or 10% for gut and muscle) and protein precipitation allowed to go to completion. The protein was spun down, washed with water and dried at $100\text{--}120^\circ$ overnight.

This material was used to determine 'protein-bound' activity. The combined supernatants from the protein precipitation were then shaken with 1 g. of charcoal (cf. Dalgliesh, 1955a). After time had been allowed for adsorption of aromatic substances the charcoal was filtered off, washed with water and dried and the activity determined. This value was taken to represent 'non-protein-bound aromatic' activity. It should be emphasized that this fractionation is crude. The 'protein-bound' activity may well include substances co-precipitated with the protein, and the 'non-protein-bound aromatic' activity may well include peptides or proteins not precipitable by heat and trichloroacetic acid.

The other organs were in general treated in the same way as the liver. The gut contents were thoroughly washed out before homogenization, and labelled amino acid which had not been absorbed was thereby removed. With gut it was particularly difficult to get an optically clear supernatant after the protein precipitation. For small organs, such as brain and lung, only total activity was determined. The bone was dried and powdered. The respiratory CO_2 was counted as BaCO_3 .

In the shortest experiments no urine or faeces were usually produced. In the longer-term experiments the activity lost in urine and faeces was small, and these were therefore ignored. The total activity recovered in all organs and respiratory CO_2 was assessed, and values given in the paper are percentages of this recovery value. This was done to eliminate errors due to incomplete administration by stomach tube, or to incomplete absorption, the gut contents being thoroughly washed out and discarded.

Activity determination. Samples were counted at infinite thickness on 1, 2 or 5 sq.cm. planchets, a thin mica end-window Geiger tube and an automatic counter being used. Counts were continued to a standard error of $\pm 5\%$ for the lowest activities and $\pm 3\%$ for higher activities. For low-activity samples, determinations of background were made for each planchet in the appropriate position of the turntable of the automatic counter, before the planchet was made up with the labelled material. The following figures, obtained in the 3 hr. tyrosine experiment, show the orders of activity encountered. $5\text{--}6\text{ }\mu\text{C}$ of the amino acid were given. The total activity recovered in tissues and respiratory CO_2 was 86.9% of this amount. (All percentages in Figs. 1-4 relate to the amount recovered.) The counts in the 'protein bound' fraction of gut, liver, muscle and blood were respectively 284, 232, 22 and 105 counts/min./sq.cm. disk at infinite thickness. Under the same counting conditions a plastic standard equivalent to $1.02\text{ }\mu\text{C}/\text{g.}$ (as BaCO_3) gave 716 counts/min. Background was in the region of 7-10 counts/min.

RESULTS

The experiments were largely dominated by the small amounts of labelled amino acids available. These dictated (a) that animal variation must be ignored, (b) that only single doses could be used, of not more than $6\text{ }\mu\text{C}$ for tyrosine or $2\text{--}4\text{ }\mu\text{C}$ for phenylalanine and tryptophan, for each time interval examined. The resultant low levels of activity make the accuracy in certain cases low, the more so as it was necessary to use relatively insensitive infinite-thickness counting techniques.

Moreover, many assumptions have had to be made. Thus we have assumed that administration of the amino acid under our experimental conditions approximates to the conditions obtaining during normal physiological intake. As the amount of amino acid administered did not exceed 1 mg. it is unlikely that an appreciable amino acid imbalance was induced. In order to eliminate difficulties due to incomplete administration or absorption, the activities found in the various tissues are expressed as percentages of the total activity recovered. Moreover, we have had to assume that different tissues, e.g. muscle, gut and liver, are each homogeneous. It is thus obviously unjustifiable to draw conclusions of more than a qualitative nature. But as experiments were carried out under comparable conditions we feel that with those organs containing an appreciable proportion of the administered activity the results are sufficiently accurate to permit comparisons of the metabolism of the three amino acids investigated.

Some indication of the relative overall rates of degradation of the three amino-acids is obtained by comparing the rates of appearance of activity in the respiratory CO_2 , as shown in Fig. 1. The rate

Fig. 1. Proportion of total absorbed activity appearing in the respiratory CO_2 . ●, Tyrosine; ×, phenylalanine; ○, tryptophan. Each point derived from a different single animal.

of breakdown of tyrosine is rapid and is about double that of phenylalanine. Conversion of phenylalanine into tyrosine is the initial step of phenylalanine degradation, and this hydroxylation reaction must therefore be rate-limiting. The overall rate of breakdown of tryptophan is similar to that of phenylalanine. In normal degradation of tryptophan the alanine side-chain is lost and C_2 of the indole ring is liberated as formate. It seems unlikely that these degradation products can alone account for the observed rate of appearance of activity in respiratory CO_2 . Thus, 1 hr. after tryptophan has been given, more than half the activity is

in the 'protein-bound' or 'non-protein-bound aromatic' fractions. It is difficult to say to what extent the 'protein-bound' fraction represents tryptophan (e.g. it probably also includes alanine from side-chain scission), and similarly the 'non-protein-bound aromatic' fraction may have undergone some degradation. But it is not unreasonable to assume that at this time about half the tryptophan molecules are still intact. Of the activity originally in the remaining molecules one-quarter has therefore appeared as CO_2 after 1 hr. We consider this to be strong evidence that a ready route for degradation of the aromatic ring must exist.

Fig. 2. Proportion of total absorbed activity appearing in muscle. ●, Total activity; ×, 'protein-bound' activity; ○, 'non-protein-bound aromatic' activity. Each point derived from a different single animal.

The organs containing much the largest proportions of the activity were muscle (Fig. 2), gut (Fig. 3) and liver (Fig. 4).

The results obtained with muscle in the tyrosine experiments are striking. The total activity rises to a high value and then falls, while protein activity rises more slowly. Less than one-quarter of the activity in muscle at 15 min. is protein-bound. Of

the non-protein-bound activity almost half (corresponding to about 15% of the total body activity) is present as non-aromatic substances. Obviously breakdown of the aromatic ring has been exceedingly rapid to allow such a large proportion of non-aromatic material to be present in the muscle after so short a time.

Similar changes were found in muscle in the phenylalanine experiments. There seems to be a higher proportion of the administered activity in muscle protein than with tyrosine, but this may represent both phenylalanine and derived tyrosine.

Fig. 3. Proportion of total absorbed activity appearing in gut. ●, Total activity; ×, 'protein-bound' activity; ○, 'non-protein-bound aromatic' activity. Each point derived from a different single animal.

The high values in the 'protein-bound' fraction after even short periods suggest that some of the activity may be coprecipitated with the protein without necessarily being in peptide combination. The general picture with tryptophan resembles that with phenylalanine. With all three amino acids there is a high initial non-protein-bound activity in the muscle which rapidly decreases, while protein incorporation more slowly increases. This situation is analogous to that found with glycine in rabbit muscle by Henriques, Henriques & Neuberger (1955), who have discussed its significance.

With gut (Fig. 3) and liver (Fig. 4) the results with the three amino acids are again similar. Free amino acid (as represented by 'non-protein-bound aromatic' activity) is low in all except the shortest time intervals examined; this agrees with the findings of Tallan, Moore & Stein (1954), who have shown that in the cat free aromatic amino acids are present in the tissues in only small amounts. The relatively large proportion of the total body activity appearing in gut supports the existing evidence on the high-metabolic activity of the gut wall (see Fisher, 1954, for references; and Winnick, Friedberg & Greenberg, 1948, for similar evidence with DL- $[\beta\text{-}^{14}\text{C}]$ tyrosine). It will be seen that a larger proportion of activity appears in the gut with tryptophan than with phenylalanine or tyrosine. We consider that this, taken with the evidence from Fig. 5 discussed below, indicates that the transfer process through the gut wall is

Fig. 4. Proportion of total absorbed activity appearing in liver. ●, Total activity; ×, 'protein-bound' activity; ○, 'non-protein-bound aromatic' activity. Each point derived from a different single animal.

slower with tryptophan. In both gut and liver even at short time intervals the greater part of the total activity is accounted for by the 'protein-bound' fraction. It may be that in the early stages the 'protein-bound' fraction contains activity that is coprecipitated with protein rather than true protein, but it should be emphasized that the curves in Figs. 2-4 represent the proportion of total activity in the particular tissue rather than the absolute amount of labelled amino acid present. The scatter of the experimental points in Figs. 2-4 is probably to a large extent due to animal variation, each point representing only a single animal.

The remaining organs individually accounted for only a small proportion of the body content of activity. Surprisingly in all experiments bone was one of the more active organs (up to 4% of the total body activity), and this presumably reflects the metabolic activity of the marrow. Spleen, brain, heart and lung usually contained 0.5–2.0% each, and kidney somewhat more. The blood activity was particularly interesting in the experiments with tyrosine. At 15 min. the 'non-protein-bound

were probably not *p*-hydroxyphenyl-pyruvic or -acetic acids. Similar high activity at 15 min. was not found in the experiments with phenylalanine or tryptophan.

In Fig. 5 the specific activities of the proteins of liver, gut and muscle are compared with the activities of blood protein at the same times. It will be seen that the results for the three amino acids are generally similar. The liver and gut protein were more active than blood protein at all time intervals examined, but the ratio decreased rapidly in the early stages. Muscle protein was at all times less active than blood protein. The unusually high ratio of gut to blood protein at 15 and 30 min. in the tryptophan experiments, together with the evidence from Fig. 3, suggests that tryptophan remains longer in the gut wall than does either phenylalanine or tyrosine, i.e. the transfer of tryptophan through the gut wall may be a slower process.

DISCUSSION

So far as we are aware no previous study of the overall metabolism of uniformly ^{14}C -labelled aromatic amino acids has been made. By the use of uniformly, rather than specifically, labelled amino acids, it should be possible roughly to estimate overall metabolic rates. The limits of accuracy imposed by available materials and apparatus in the present work do not justify quantitative deductions; however, as experiments were carried out under similar conditions, it is thought that qualitative comparisons are valid.

Only small proportions of the phenylalanine and tyrosine ingested by a normal mammal are excreted as aromatic substances. The benzoic acid moiety of hippuric acid and the phenylacetic acid moiety of phenylacetylglutamine are probably derived at least partly from dietary phenylalanine either by direct conversion in body tissues (Schreier, Altman & Hempelman, 1954) or by the action of the bacterial flora of the gut. (The remainder may come from other aromatic substances in the vegetable matter in the diet.) Small amounts of aromatic precursors or degradation products of adrenaline, melanin and the thyroid hormones may also be present, together with traces of phenol and other products of bacterial action. But the greater part of the normal intake of phenylalanine and tyrosine is converted into non-aromatic substances. This is easily explained by enzymic experiments (e.g. Knox & Le May-Knox, 1951; see also the detailed review by Dalglish, 1955b) which show that phenylalanine is converted into tyrosine, which is then degraded to acetoacetic and fumaric acids, both substances of high-metabolic reactivity. The great potential capacity of the body for tyrosine breakdown is well shown by the

Fig. 5. Ratios of specific activities of 'protein-bound' fractions of muscle, gut and liver to specific activity of 'protein-bound' fraction of blood. ●, Gut; x, liver; ○, muscle.

aromatic' fraction of blood contained 16.5% of the total body activity, but this had dropped to 1.2% at 30 min. The charcoal carrying the 15 min. 'non-protein-bound aromatic' fraction was eluted with aqueous phenol (cf. Dalglish, 1955a), the eluate was concentrated, and run on a paper chromatogram with butanol-acetic acid-water (4:1:5, by vol.; Partridge, 1946) as solvent, and a radioautograph made of the resulting chromatogram. Three radioactive substances were present of R_f values 0.35 (corresponding to authentic tyrosine), 0.76 and 0.86. The spot of R_f 0.76 was weak, but that of R_f 0.86 was appreciably more intense than the tyrosine spot. We were unable to identify either of the fast-running substances with the limited amounts of material available, but they

experiments of Abderhalden (1912), who himself took 150 g. of tyrosine without causing abnormal excretion of aromatic metabolites.

The case of tryptophan is quite different, as can be seen by considering the known degradative pathways. A detailed discussion of the elucidation of these pathways has been given elsewhere (Dalglish, 1955*b*) and can be consulted for further references.

The major pathway of tryptophan degradation is considered to be that leading to nicotinic acid and its derivatives. This pathway is summarized in Fig. 6. In normal circumstances there is a small

occurs. However, the known ready exchange of free nicotinamide with the nicotinamide moiety of the pyridine nucleotides (Zatman, Kaplan & Colowick, 1953) suggests that incorporation and release of nicotinamide normally occur by such an exchange process.

The normal metabolism of kynurenine has been discussed in an earlier paper (Dalglish & Tekman, 1954), in which it was shown that by far the greater part is converted into hydroxykynurenine. As formylkynurenine gives kynurenine very readily, its fate can be regarded as ultimately similar. The normal fate of 3-hydroxykynurenine is to be split

Fig. 6. Generally accepted major pathway for tryptophan degradation via nicotinic acid. Interrupted arrows represent side-reactions.

excretion of unchanged tryptophan and of nicotinic acid derivatives, but none of the other substances shown in Fig. 6 is excreted in appreciable amount. Presumably, therefore, at least one of the substances shown in Fig. 6 must be degraded to non-aromatic compounds. If nicotinic acid or nicotinamide is administered to any of a wide variety of species there is very little breakdown of the aromatic ring; instead, conjugation reactions of various types take place (cf. reviews: Kodicek, 1951; Dalglish, 1955*b*). The same applies to quinolinic acid. It is therefore reasonable to infer that the pyridine compounds are not the normal precursors of non-aromatic substances. Moreover, the discrepancy probably cannot be accounted for by incorporation into pyridine nucleotides, as in the steady-state neosynthesis of pyridine nucleotide should be balanced by an equivalent breakdown of preformed material. There is a possibility that breakdown of pyridine nucleotide involves opening of the pyridine ring before scission into smaller fragments, so that aliphatic substances result from this portion of the molecule when degradation

by the enzyme kynureninase to give 3-hydroxyanthranilic acid. The side reactions of kynurenine and 3-hydroxykynurenine are unlikely to account for more than a small proportion of the ultimate degradation of tryptophan to aliphatic compounds. For example, if anthranilic acid is administered to an animal it is almost entirely excreted free or as conjugates (Charconnet-Harding, Dalglish & Neuberger, 1953; Williams, 1949) and very little ring scission occurs. Kynurenic acid appears to be metabolically almost inert. Xanthurenic acid is not normally excreted, and though there is evidence that it can be degraded *in vivo* (Weitzel, Buddecke, Strecker & Roester, 1954; Kotake, Kotake & Inouye, 1954) it seems unlikely that such degradation is a rapid process that could account for more than a small proportion of the tryptophan degraded. Many of these intermediates can be excreted on giving a large dose of tryptophan. But this is probably largely due to adaptive increase in tryptophan peroxidase-oxidase, which converts tryptophan into formylkynurenine, without similar increase in the amounts of subsequent

enzymes (cf. Dalglish & Tekman, 1954, for discussion). The amounts of tryptophan causing such abnormal excretion of intermediates are of the order of 100 or more times the 'physiological' amounts used in the present experiments.

The only remaining substance in the 'nicotinic acid pathway' of tryptophan metabolism is 3-hydroxyanthranilic acid. This is indeed a potential aliphatic precursor and is considered in more detail below.

A second pathway of tryptophan degradation is by way of 5-hydroxytryptophan, 5-hydroxytryptamine (enteramine, serotonin) and 5-hydroxyindolylacetic acid (for collected references see Dalglish, 1955b). Of these substances 5-hydroxytryptophan appears to be completely converted into 5-hydroxytryptamine. Very small amounts of hydroxytryptamine and derivatives are excreted (Bumpus & Page, 1955) and the major part is converted into hydroxyindolylacetic acid, which appears to be largely excreted as such (Erspamer, 1955; Page, Corcoran, Udenfriend, Szoedsma & Weissbach, 1955). Quantitative evidence (Erspamer, 1955) suggests that this pathway can account for only a small amount of the total tryptophan degraded (10–15 mg./day in man, compared with an intake of the order of 500 mg.).

There remain various routes for degradation of tryptophan by the bacterial flora of the gut. These are summarized in Fig. 7. The metabolites shown in

Fig. 7. Degradation of tryptophan by the bacterial flora of the gut.

Fig. 7 themselves account for a small amount of ingested tryptophan, but none appears to be a likely precursor of non-aromatic substances. Thus, administration of indolylacetic acid to an animal results in almost quantitative excretion of the free acid and its glycine conjugate, indolylaceticuric acid (Ewins & Laidlaw, 1913; Schayer, Wu, Smiley & Kobayashi, 1954).

The experimental results described in this paper

show that the rate of degradation of tryptophan is comparable with that of phenylalanine. It seems to be considerably greater than can be accounted for by our present knowledge of tryptophan metabolism. Various possibilities exist. First, there may be an unknown pathway or pathways of tryptophan degradation. The 5-hydroxytryptamine pathway is of remarkably recent discovery in spite of its physiological importance, and the existence of unknown pathways is by no means excluded. However, we consider it unlikely that a pathway accounting for a large part of the tryptophan normally degraded still remains undiscovered. Secondly, there may be a slow 'leakage' to non-aromatic compounds from several aromatic intermediates in the various tryptophan-degradative pathways. Although such 'leakage' may well contribute to tryptophan degradation we consider that it is unlikely to account for the comparatively rapid appearance of radioactivity in the respiratory CO_2 observed in the present experiments. Thirdly, an intermediate already known may be degraded by more than one route. There are two possible intermediates: the pyridine nucleotides—which we have discussed above—and 3-hydroxyanthranilic acid.

After giving the comparatively large amount of 600 mg. of 3-hydroxyanthranilic acid to a rabbit, Bray, Thorpe & Wood (1951) could account for only 13% of the dose as aromatic substances. It is therefore evident that ready breakdown of the aromatic ring occurs. In the intact rat 3-hydroxyanthranilic acid is converted into quinolinic acid to the extent of 8–15% as well as to appreciably smaller amounts of nicotinic acid and derivatives (Henderson, 1949). On the other hand, in rat-liver homogenates conversion of 3-hydroxyanthranilic acid into quinolinic acid is almost quantitative (70–100%; Bokman & Schweigert, 1950). Some transformation must therefore occur in the intact animal which does not occur in liver homogenates, presumably owing to inactivation of the appropriate enzyme. It seems to have been assumed that this other reaction is the formation of nicotinic acid, but the comparatively small amount of nicotinic acid formed when hydroxyanthranilic acid is given to an intact animal makes this unlikely, since it is known (as mentioned above) that most of the nicotinic acid formed is excreted as free or conjugated pyridine derivatives. It therefore seems likely that there is another, and major, pathway for hydroxyanthranilic acid degradation in the intact animal that allows formation of non-aromatic substances. Some conversion of hydroxyanthranilic acid into a red product can occur in certain liver preparations (Viollier & Süllmann, 1950), but this is probably an artifact, and may well be due to oxidation of hydroxyanthranilic acid

to phenoxazine derivatives analogous to those arising from hydroxykynurenine in insect eye-pigment formation (e.g. Butenandt, Schiedt & Bieker, 1954).

It is now clear, particularly from the enzymic work of Wiss (1954, 1955), that normal metabolism of 3-hydroxyanthranilic acid involves ring scission between carbons 3 and 4 to give an acroleinfumaric acid derivative (I, Fig. 8). It is also clear (for collected references see Dalgliesh, 1955b) that quinolinic acid formation from hydroxyanthranilic acid is a side reaction occurring by non-enzymic ring closure of an intermediate in nicotinic acid formation.

quinolinic acid if β -decarboxylation of the imino-acid (II) occurred, may favour the carboxyl analogues of (IV) and (V). It is possible to envisage several schemes for conversion of compounds such as (IV) and (V) into substances likely to have a high metabolic-turnover rate, but their investigation must await enzymic experiments. A pathway such as suggested here could explain the comparatively rapid degradation of tryptophan in the intact animal; and if the appropriate enzymes are more labile than, or more easily separated from, those bringing about ring opening of hydroxyanthranilic acid, the much greater proportion of

Fig. 8. Partly hypothetical scheme for the degradation of 3-hydroxyanthranilic acid.

The acroleinfumaric acid derivative (I) is most unlikely to be stable. Tautomerism to the imino acid derivative (II, Fig. 8) allows conversion into the acroleinmaleic acid derivative (III) which would almost certainly cyclize spontaneously to quinolinic acid. Nicotinic acid might be formed by decarboxylation of the α -carboxyl group of (I), (II) or (III) before cyclization, and this is presumably an enzymic process. It is now suggested that either an appreciable part of hydroxyanthranilic acid metabolism can occur via the imino acid (II) to the keto acid (IV), in which the β -carboxyl group is doubly activated and will be readily lost to give the acroleinpyruvic acid (V), or the aldehyde group in (II) is first oxidized to a carboxyl group and similar reactions occur. The apparent absence of formation of picolinic acid, which might be expected to arise in an analogous way to

Fig. 9. Generalized scheme for breakdown of 3-hydroxyanthranilic acid.

hydroxyanthranilic acid converted into quinolinic acid in liver preparations compared with the intact animal can be readily explained. It is therefore proposed that it is via 3-hydroxyanthranilic acid that a larger part of tryptophan degraded *in vivo* reaches aliphatic metabolic pools, and that the metabolism of 3-hydroxyanthranilic acid can be summarized as shown in Fig. 9.

SUMMARY

1. Adult rats starved for 16 hr. have been given solutions of uniformly ^{14}C -labelled L-phenylalanine, L-tyrosine or L-tryptophan by stomach tube. The amount given (<1 mg.) was considered small enough to represent an approximation to physiological conditions. The radioactivity in respired carbon dioxide and in different organs was determined after varying intervals.

2. The rate of liberation of the label in the respiratory carbon dioxide showed that all three amino acids can be rapidly converted into non-aromatic substances of high metabolic activity. The overall rates of degradation of phenylalanine and tryptophan were similar, and about one-half that of tyrosine. Hydroxylation to tyrosine is thus the rate-limiting step in normal phenylalanine metabolism.

3. The rate of appearance of activity in whole muscle, liver and gut, and in the 'protein-bound' and 'non-protein-bound aromatic' fractions of these organs, is reported. At all times examined the specific activity of liver and gut protein exceeded that of blood protein, but muscle protein was less active.

4. In the existing state of our knowledge of tryptophan metabolism no pathway is known which appears to account for the observed comparatively rapid degradation of tryptophan. A survey of the various possibilities suggests that, unless a major pathway of tryptophan metabolism remains undiscovered, degradation is most likely to occur via 3-hydroxyanthranilic acid which, it is suggested, gives rise not only to nicotinic and quinolinic acids but also to non-nitrogenous substances with a high metabolic-turnover rate. Initial stages in the formation of such substances are proposed.

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