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The Distribution of L-Asparagine Synthetase in the Principal Organs of Several Mammalian and Avian Species

By HARRY A. MILMAN and DAVID A. COONEY
Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014, *U.S.A.*

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A survey was conducted of the distribution of L-asparagine synthetase and of L-asparaginase in the principal organs of representative mammals and birds. Although a radiometric assay was used as a routine, several additional criteria, including enzymic and chromatographic ones, were used to verify that the product of the synthetase was L-asparagine. Recoveries of exogenous L-asparagine were assessed in the presence of a number of mouse organs and found to be about 85%. In addition, evidence is presented for the existence in mouse liver of a thermolabile activity capable of destroying L-asparagine and stimulated by high concentrations of NH_4^+ ions. Of the organs surveyed, pancreas was generally found to synthesize L-asparagine at the most rapid rate, whereas extracts of liver catalysed the decomposition of this amide at the greatest velocity. Of the species studied, guinea pig had the highest activities of pancreatic L-asparagine synthetase and also of hepatic L-asparaginase. The pancreas of mouse and ox also were good sources of L-asparagine synthetase.

L-Asparagine has been shown to be synthesized in a variety of organisms: in yeast (Al-Dawody, 1961) from L-aspartate by the phosphorolytic cleavage of ATP with NH_4^+ ions as precursors of the amide, in certain bacteria (Ravel *et al.*, 1962) and mammals (Horowitz *et al.*, 1968) by the pyrophosphorolytic cleavage of ATP, with either NH_4^+ ions or L-glutamine as amide donor, and in certain plants (Ressler *et al.*, 1969) from β-cyano-L-alanine without the direct participation of an energy donor.

For some time it has been known that experimental tumours sensitive to the enzyme L-asparaginase exhibit low or undetectable activities of L-asparagine synthetase. Resistant variants acquire an increased ability to synthesize L-asparagine attributable to elevated concentrations of L-asparagine synthetase in the neoplastic tissue. Since inhibition of L-asparagine synthetase should confer sensitivity on the formerly resistant cell, our laboratory has been searching for suitable inhibitors of the enzyme. During our studies on the biological effects of several such agents, we considered that it would be important to know whether the potential inhibitors were disturbing the metabolism of L-asparagine in normal organs to the same extent as in resistant tumours. Such a comparative investigation requires some systematic knowledge of the ability of various tissues to synthesize L-asparagine. To date, however, only two rather limited surveys of the distribution of L-asparagine synthetase in mammalian organs have been published. In the study which first linked the resistance of experimental tumours to L-asparaginase with augmented activities of tumoural L-asparagine synthetase, Horowitz *et al.* (1968) found that high-speed supernatants of brain and testis of the mouse exhibited the highest capacity to synthesize L-asparagine of the six organs studied. Holcenberg (1969) surveyed the tissues of the guinea pig, a species whose plasma contains L-asparaginase, and reported that the small intestine contained the richest concentration of L-asparagine synthetase, followed in decreasing order by the spleen, liver, stomach and large intestine. Milman *et al.* (1972) reported that typical L-glutamine-dependent L-asparagine synthetase was absent from the principal organs of several fishes. In the present paper, a more extensive and systematic survey of the distribution of this enzyme in several mammalian and avian species is presented.

Experimental

Materials

Enzymes. L-Asparaginase (EC 3.5.1.1) from *Escherichia coli* (340 units/mg of protein) was purified at the Merck Institute for Therapeutic Research, West Point, Pa., U.S.A., and provided by the Drug Research and Development Branch of the National Cancer Institute. L-Aspartate 4-decarboxylase (EC 4.1.1.12) was purified (Tate & Meister, 1968) and provided by Dr. Suresh Tate of Cornell University, School of Medicine, New York, N.Y., U.S.A. The enzyme exhibited a specific activity, with L-aspartate

as substrate, of 77 units/mg of protein, and was stored in individual 0.1ml portions at −87°C in 50% (v/v) glycerol (pH5.0), 20μM with respect to pyridoxal phosphate. This solution contained 300 units/ml. Malate dehydrogenase (EC 1.1.1.37) (720 units/mg of protein) and L-glutamate–oxaloacetate transaminase (EC 2.6.1.1) (190 units/mg of protein) were purchased from Boehringer (Mannheim) Corp., New York, N.Y., U.S.A.

Radiochemicals. L-[4-^{14}C]Aspartate (specific radioactivity 26μCi/μmol) was the product of Biochemical and Nuclear Corp., Burbank, Calif., U.S.A. Purity of the substrate was assessed in three ways: by high-voltage paper electrophoresis (on Whatman 3MM paper) at 3000V, in 0.05M-sodium citrate buffer, pH6.0, containing 0.05M-$ZnSO_4$; by automatic amino acid analysis with the Jeolco AR50 amino acid analyser; and by quantitative 4-decarboxylation with L-aspartate 4-decarboxylase (Horowitz & Meister, 1972). The first two methods revealed any possible impurities having a different charge from that of L-aspartate, and the third method, taken in conjunction with the other two, permitted the detection of the presence of any D-isomer, inasmuch as the reagent enzyme is specific for the L-isomer. L-[U-^{14}C]Asparagine (specific radioactivity 151μCi/μmol) was the product of Amersham Corp., Silver Spring, Md., U.S.A. Purity (99%) of this compound was assessed by paper electrophoresis and amino acid analysis as described above, and also by hydrolysis with L-asparaginase (2 units/ml) and subsequent treatment with L-aspartate 4-decarboxylase. The $^{14}CO_2$ obtained by the latter method represented one-quarter of the total radioactivity present in the vessel, owing to the fact that the amino acid was isolated from the protein of algae that had been grown with nearly pure $^{14}CO_2$ as the sole carbon source. Recoveries with L-aspartate 4-decarboxylase were compared with those obtained after treatment of L-[U-^{14}C]asparagine with L-asparaginase (2 units/ml) and 'decarboxylation mixture' (see under 'Methods') and found to be identical.

Chemicals. L-Asparagine was purchased from Schwartz–Mann Biological Research, Rockville, Md., U.S.A., and stored in a desiccator over $CaCl_2$ until used. NADH was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.; L-glutamine and 2-oxoglutarate were the products of Calbiochem, Los Angeles, Calif., U.S.A. All other chemicals were reagent grade.

Vessels. Eppendorf 1500μl disposable plastic centrifuge vessels, from Brinkman Instrument Co., Silver Spring, Md., U.S.A. were used throughout.

Tumour. Leukaemia 5178Y was made resistant to L-asparaginase by treating BDF_1 mice bearing the native or sensitive form of the tumour with daily intraperitoneal injections of 0.75unit of L-asparaginase for the first 6 days of tumour growth. On the 7th day, 10^6 leukaemic cells were transplanted to a second generation of BDF_1 mice and daily intraperitoneal injections of 15 units of L-asparaginase were administered. On the 7th day, 10^6 leukaemic cells of the second generation were transplanted to a third generation of BDF_1 mice and 30 units of L-asparaginase were injected intraperitoneally on days 1–6. On the 7th day, 10^6 cells of the third generation were transplanted subcutaneously to a new set of BDF_1 mice and tumours were allowed to grow subcutaneously until day 10. Resistance to L-asparaginase was maintained by challenging the tumour with intraperitoneal injections of 30 units of L-asparaginase on days 1–6.

On day 10, the mice were killed by neck dislocation and the solid tumours removed and homogenized (1:9, w/v) in 0.1M-Tris–HCl buffer, pH7.5, containing 0.5mM-EDTA disodium salt and 1mM-dithiothreitol. The homogenate was then centrifuged for 10min at 25000*g* and the supernatant dispensed in 200μl batches into Eppendorf 1500μl disposable plastic centrifuge vessels and stored at −87°C until use.

Organ samples. Organs of adult chicken, pig, cat, turkey and sheep were purchased from Pel-Freeze Biologicals Inc., Rogers, Ark., U.S.A. They had been frozen immediately after excision. Organs from other mammalian species (see Table 1) were removed in our laboratory, homogenized and stored at −87°C until the time of assay.

Methods

Homogenization of organs. Organ samples were homogenized at 4°C in 9vol. of 0.1M-Tris–HCl buffer, pH7.6, containing 1mM-dithiothreitol and 0.5mM-EDTA. The homogenates were transferred to Eppendorf 1500μl disposable plastic centrifuge vessels and centrifuged in the Eppendorf Zentrifuge at 12000*g* for 3min. The supernatant was stored at −87°C until assay.

Procedure for measuring L-asparagine synthetase. A radioactive 'cocktail' was prepared as follows: 2ml of 0.02M-ATP, 0.04M-L-glutamine and 0.02M-$MgCl_2$ in 0.5M-Tris–HCl buffer, pH7.5, was added to 50μCi of dry L-[4-^{14}C]aspartate, so that 5μl of the 'cocktail' contained 0.1μmol of L-glutamine, 0.05μmol of $MgCl_2$, 0.05μmol of ATP, 0.0048μmol of L-aspartate and 277500d.p.m.

(*a*) Incubation: synthesis of L-asparagine. A portion (5μl) of the 12000*g* supernatant of a 1:9 (w/v) organ homogenate was added in triplicate to Eppendorf 1500μl conical centrifuge vessels containing 5μl of the radioactive 'cocktail' and the two solutions were mixed by a brief (approx. 5s at 12000*g*) centrifugation in the Eppendorf Zentrifuge. The closed vessels were then placed at 37°C for 30min on an Eppendorf automatic hot-plate, after which the reaction was terminated by heating the vessels at 95°C for 5min.

In this incubation step, the following reaction takes place:

$$\text{L-[4-}^{14}\text{C]Aspartate} + \text{L-glutamine} \xrightarrow[\substack{\text{Mg}^{2+}\\ \text{L-asparagine}\\ \text{synthetase}}]{\text{ATP}} \begin{array}{l}\text{L-[4-}^{14}\text{C]asparagine}\\ +\text{L-glutamate}\\ +\text{AMP}\\ +\text{PP}_i\end{array} \quad (1)$$

After the vessels had cooled, 1 unit of L-asparaginase in a volume of 5μl was added to the third vessel of each group. This step, which served to hydrolyse L-[4-^{14}C]asparagine to L-[4-^{14}C]aspartate, provided an internal control.

(*b*) Inactivation of unchanged L-[4-^{14}C]aspartate. An inactivation mixture was prepared by dissolving 5mg of 2-oxoglutarate, 10mg of NADH, 54 units of L-glutamate–oxaloacetate transaminase and 165 units of malate dehydrogenase in 250μl of 0.5M-Tris–HCl buffer, pH8.4. A portion (10μl) of the resulting solution was added to each vessel and driven down by a 5s centrifugation at 12000*g* in the Eppendorf Zentrifuge. The closed vessels were incubated on an Eppendorf hot-plate at 37°C for 30min, then transferred to 95°C for 10min. In the inactivation step the following reactions take place:

$$\text{L-[4-}^{14}\text{C]Aspartate} + \text{2-oxoglutarate} \xrightarrow[\text{transaminase}]{\substack{\text{L-glutamate–oxalo-}\\ \text{acetate}}} \text{[4-}^{14}\text{C]oxaloacetate} + \text{L-glutamate} \quad (2)$$

$$\text{[4-}^{14}\text{C]Oxaloacetate} + \text{NADH} \xrightarrow[\text{dehydrogenase}]{\text{malate}} \text{[4-}^{14}\text{C]malate} + \text{NAD}^+ \quad (3)$$

At this point, the principal radioactive components of the experimental vessels were L-[4-^{14}C]malate and approx. 1% of L-[4-^{14}C]aspartate, which had escaped inactivation. In the control vessels, the radioactive components were L-[4-^{14}C]aspartate and [4-^{14}C]-malate.

(*c*) Decarboxylation of residual L-[4-^{14}C]aspartate. A portion (50μl) of a 'decarboxylation mixture' [composed of 10mg of 2-oxoglutarate, 70μl of 70% (w/v) $ZnSO_4$ and 36 units of L-glutamate–oxaloacetate transaminase in 10ml of 0.66M-sodium acetate buffer, pH5.0] was added to the cooled vessels. Then the open vessels were placed on the Eppendorf hot-plate at 37°C for 60min under the hood. In this step the following reactions occur:

$$\text{L-[4-}^{14}\text{C]Aspartate} + \text{2-oxoglutarate} \xrightarrow[\text{transaminase}]{\substack{\text{L-glutamate–}\\ \text{oxaloacetate}}} \text{[4-}^{14}\text{C]oxaloacetate} + \text{L-glutamate} \quad (4)$$

$$\text{[4-}^{14}\text{C]Oxaloacetate} + \text{Zn}^{2+} \xrightarrow{\text{H}^+} {}^{14}\text{CO}_2 + \text{pyruvate} \quad (5)$$

At this point, the principal radioactive components of the experimental vessels were L-[4-^{14}C]asparagine and [4-^{14}C]malate; the principal radioactive component of the control vessels was [4-^{14}C]malate only.

(*d*) Recovery of L-[4-^{14}C]asparagine. To the open vessels containing the 'decarboxylation mixture', 2 units of L-asparaginase in a volume of 10μl were added. Immediately thereafter, 5μl of 40% (w/v) KOH was dispensed on the underside of the lid of the vessels and the covers were closed securely. The vessels were then incubated at 37°C on an Eppendorf hot-plate for 3h. In this step L-[4-^{14}C]asparagine is hydrolysed to L-[4-^{14}C]aspartate and NH_3 in the experimental vessels and the L-[4-^{14}C]aspartate so generated undergoes reactions 4 and 5 listed above.

The $^{14}CO_2$ was collected and counted for radioactivity as previously described (Cooney *et al.*, 1971).

A 5μl portion of the 25000*g* supernatant from a 1:9 (w/v) homogenate of the Leukaemia 5178Y tumour rendered resistant to L-asparaginase (see above) was always included as standard in each assay.

Assay procedure for L-asparaginase. A 200μl portion of the 12000*g* supernatant of a 1:9 (w/v) organ homogenate was added to a 1500μl Eppendorf conical vessel containing 500μl of 0.1M-L-asparagine in 0.05M-Tris–HCl buffer, pH8.5; the two solutions were mixed by inversion and incubated on the Eppendorf hot-plate for 60min at 37°C. The reaction was terminated by heating at 95°C for 20min. After centrifugation at 12000*g* for 3min, the concentration of liberated L-aspartate was assessed by an enzymic spectrophotometric technique (Cooney *et al.*, 1970).

Protein determinations. Protein measurements on the 12000*g* supernatant of the crude organ homo-

genate were done by the method of Lowry *et al.* (1951), with bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill., U.S.A.) as standard.

Results and Discussion

Verification that L-*asparagine is the product synthesized*

That L-asparagine was the product of the reaction catalysed by the mouse pancreatic L-asparagine synthetase was verified by analysis of a typical incubation mixture on the Jeolco AR50 amino acid analyser. Lithium citrate buffers were used for the resolution of L-asparagine, essentially as described by Benson *et al.* (1967). Fig. 1 shows that the radioactive peak in the experimental sample corresponds to authentic radioactive L-asparagine in the standard sample. Further, in the control sample, to which L-asparaginase has been added after the incubation period, no radioactive L-asparagine is observed. Instead, the radioactive L-aspartate peak is increased in area. It can be concluded that it is L-asparagine that has been synthesized by the enzyme present in the crude pancreatic homogenate used.

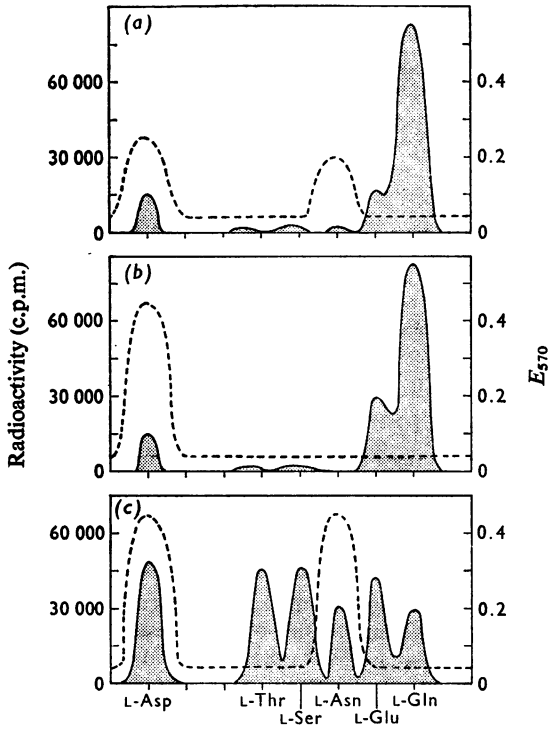

Fig. 1. *Amino acid analysis of a mouse pancreatic synthetase incubation mixture*

Twenty typical incubation mixtures of mouse pancreatic synthetase were pooled, then divided into two equal parts. To one sample was added 10μl of *E. coli* L-asparaginase solution (200 units/ml); this was the control sample (*b*). To the other sample was added 10μl of water; this was the experimental sample (*a*). After 30min both samples, as well as a standard (*c*) containing labelled and unlabelled amino acids, were loaded on the amino acid analyser (see the Experimental section). Dotted lines represent radioactivity; darkened areas represent E_{570}.

Recovery of exogenous L-*asparagine in the assay system*

To test the efficiency of the assay system, 5μl of the 12000*g* supernatant from a 1:9 (w/v) organ homogenate of the brain, kidney, liver, testis, pancreas, spleen or lung of the mouse, or 5μl of the 25000*g* supernatant from a 1:9 (w/v) Leukaemia 5178Y tumour homogenate, were incubated with 0.0015μmol of L-[U-^{14}C]asparagine for 30min at 37°C, then heated at 95°C for 5min. L-[U-^{14}C]-Asparagine was used for exogenous recovery measurements instead of L-[4-^{14}C]asparagine in view of the significantly greater purity of the uniformly labelled product. Subsequently the inactivation and decarboxylation steps were carried out as previously described and the $^{14}CO_2$ generated, representing one-quarter of the radioactivity of L-[U-^{14}C]asparagine, was collected. Some 85% of the theoretical radioactivity associated with the fourth carbon atom of the L-[U-^{14}C]asparagine was recovered from those vessels to which supernatant of the pancreas, spleen, kidney, testis, lung or brain homogenates were added; 75% of the theoretical radioactivity was recovered from vessels to which supernatant from the liver homogenate was added. Apparently, this 10% decrease in recovery of L-[U-^{14}C]asparagine from the liver samples was due to the presence of endogenous L-asparaginase. L-Asparagine recovery from liver samples was then studied in the presence of 0.2M-$(NH_4)_2SO_4$, a salt found to inhibit mouse hepatic L-asparaginase by over 90% via product inhibition. Under these conditions, recovery of L-[U-^{14}C]-asparagine from liver samples unexpectedly was 30% lower than in the absence of $(NH_4)_2SO_4$. When recovery of L-[U-^{14}C]asparagine was performed from 12000*g* liver supernatant that had been previously heated at 56°C for 10min, no appreciable difference in recovery was observed between samples assayed in the presence or absence of 0.2M-$(NH_4)_2SO_4$, a finding which indicates that heating destroyed the activity responsible for the decrease in recovery. Recovery in the presence of 0.2M-NH_4Cl was equivalent to that obtained in the presence of 0.2M-$(NH_4)_2SO_4$. We found that 0.02M-K_2SO_4 appeared to inhibit the recovery of L-[U-^{14}C]asparagine, perhaps by inhibiting the reagent enzymes used in the assay. These results suggest the presence of an NH_4^+-dependent L-asparagine-utilizing enzyme in the 12000*g* mouse liver supernatant which is responsible for the 30%

decrease in L-asparagine recovery from liver samples in the presence of $(NH_4)_2SO_4$. It is for this reason that $(NH_4)_2SO_4$ was not used as a routine to inhibit liver L-asparaginase in the majority of the species studied. However, all samples of guinea-pig organs were assayed in the presence of 0.2M-$(NH_4)_2SO_4$, previously shown by Holcenberg (1969) effectively to inhibit L-asparaginase in that species. Our studies showed 75% inhibition of guinea-pig organ L-asparaginase by 0.2M-$(NH_4)_2SO_4$ without concurrent inhibition of L-asparagine synthetase.

When recoveries of L-[U-^{14}C]asparagine were measured from supernatants of the seven mouse organ homogenates that had been previously heated at 95°C for 10min, 85% of the theoretical radioactivity associated with the fourth carbon atom of L-[U-^{14}C]-asparagine was observed. Further, no synthesis of L-asparagine was detectable in organ homogenates heated at 95°C. In the light of these recoveries, no corrections have been made in the L-asparagine synthetase activity measurements.

Distribution of L-*asparagine synthetase and* L-*asparaginase*

The distribution of L-asparagine synthetase in the organs of several mammalian and avian species is presented in Table 1. Of the principal organs studied, pancreas invariably had the greatest ability to synthesize L-asparagine. Of the species studied, the guinea-pig pancreas contains the enzyme at highest specific activity (44.08nmol/h per mg of protein). Ox pancreas carries out the synthesis of L-asparagine *in vitro* at a vigorous rate (26.38nmol/h per mg of protein), and therefore may serve as a suitable source for the purification of mammalian L-asparagine synthetase. The finding that mouse pancreas contains a high activity of synthetase makes that species an attractive and convenient laboratory model for studies of the function of the enzyme *in vivo*.

Lower activities of L-asparagine synthetase were found in the pancreas of the chicken, turkey, sheep, rabbit, pig, monkey and man. The disproportionately low activity found in human pancreas may be explained in part by the pancreatic samples being obtained as late as 4–6h *post mortem*, so that a significant degree of proteolysis may have taken place.

Holcenberg (1969) reported that the small intestine of the guinea-pig, of all the organs studied, synthesized L-asparagine at the greatest rate (34nmol/h per mg of protein). Pancreas was not included in that survey. Our measurements in the small intestine of the guinea pig have likewise revealed high intestinal synthetase activity (17nmol/h per mg of protein), second only to the pancreas.

The brain generally had the next highest activity of L-asparagine synthetase. Species that showed a substantial cerebral synthetase activity were the rat, cat and pig. Significantly lower activity was detected in the brain of the mouse, guinea pig, rabbit, chicken, turkey, dog, ox, sheep and monkey. Our value for synthetase activity in rat brain (5.92nmol/h per mg of protein) compares favourably with that obtained by Benuck *et al.* (1970), who reported that whole adult rat brain synthesized L-asparagine at the rate of 4.7nmol/h per mg of protein.

Male and female gonads, kidney, spleen and lung were approximately equal in their concentration of the enzyme. Horowitz *et al.* (1968) reported that mouse testis synthesizes L-asparagine at a rate (13.6nmol/h per mg of protein) greater than that of brain, spleen, liver, kidney and lymph nodes. The value given in Table 1 for mouse testis is substantially lower than this. We observed that the activity of L-asparagine synthetase in mouse testis varies with age, reaching a peak at adolescence; the discrepancy in values may be due to this phenomenon.

Synthetase activity in liver samples was notably low in the present survey. As explained above, apparently in the mouse there is an NH_4^+-dependent L-asparagine-utilizing enzyme in the 12000*g* liver supernatant. Such an enzyme may also be present in the liver of some of the other species studied. Holcenberg (1969) reported that guinea-pig liver synthesized L-asparagine at the rate of 18nmol/h per mg of protein. In spite of the inhibition of endogenous L-asparaginase with $(NH_4)_2SO_4$, our studies revealed a mean L-asparagine synthesis rate of only 1.92nmol/h per mg of protein in guinea-pig liver. This lower activity may reflect the changes in liver L-asparaginase activity which occur with changes in diet, sex and age (Bonetti *et al.*, 1969), modulations that can be expected either to stimulate or to inhibit L-asparagine synthetase.

Patterson & Orr (1969) reported that rat liver synthetase activity was highest during the embryonic stages (1.16nmol/h per mg of protein at −3 days) and declined continuously until at least 60 days *post partum* to a value of 0.16nmol/h per mg of protein. In the present survey, the mean adult rat liver synthetase activity (0.66nmol/h per mg of protein) conforms well with the above findings.

On the basis of extensive experiments with the isolated perfused rat liver, Woods & Handschumacher (1971) concluded that the liver is a regulatory site for the homoeostasis of L-asparagine in plasma in the rat. These authors suggest that hepatic L-asparagine synthetase, acting in tandem with hepatic L-asparaginase, may control the efflux of L-asparagine into the general circulation. In this regard it is notable that our measurements of rat liver synthetase activity are low, in absolute terms (0.66nmol/h per mg of protein). It is possible that L-asparagine is synthesized within the liver by some route other than by an L-glutamine- or NH_4^+-dependent enzyme system or that the system requires intact cells for efficient operation.

A number of factors, other than the absence of L-asparagine synthetase, might have operated to pre-

Table 1. *Distribution of* L-*asparagine synthetase and* L-*asparaginase in several organs of mammalian and avian species*

L-Asparagine synthetase and L-asparaginase activities and protein were measured in the 12000*g* supernatant of the 1:9 (w/v) organ homogenates of the species listed in the Table by the procedures outlined in the Experimental section. All values represent the mean (range) of five animals and ten determinations. The first row of values for each animal is the mean, with the range in parentheses, of L-asparagine synthesized (nmol/h) and the second row is the mean, with the range in parentheses, of L-asparagine hydrolysed (μmol/h).

	Pancreas		Liver	
Species	Activity/g of tissue	Activity/mg of protein	Activity/g of tissue	Activity/mg of protein
Guinea pig	3547.0 (3222.0–3953.0)	44.08 (41.36–44.26)	264.0 (189.0–357.0)	1.92 (1.47–2.38)
	18.7 (16.6–20.4)	0.30 (0.19–0.38)	95.8 (84.0–109.8)	0.91 (0.88–0.96)
Cat	1208.0 (610.0–2126.0)	31.35 (13.55–50.88)	20.0 (19.0–30.0)	0.23 (0.16–0.32)
	3.4 (2.4–5.4)	0.05 (0.03–0.06)	12.8 (11.2–13.7)	0.14 (0.12–0.15)
Ox	1203.0 (709.0–1676.0)	26.38 (21.35–33.92)	141.0	1.41
	4.5 (3.0–5.4)	0.06 (0.05–0.07)	3.6	0.03
Mouse	1723.0 (1535.0–1833.0)	24.58 (19.75–35.53)	9.0 (7.0–11.0)	0.03
	4.2 (3.6–4.8)	0.05 (0.04–0.08)	100.0 (81.6–115.8)	0.95 (0.87–1.08)
Rat	1309.0 (956.0–1732.0)	19.58 (16.11–22.22)	60.0 (49.0–74.0)	0.66 (0.50–0.80)
	3.9 (3.6–4.2)	0.07 (0.06–0.08)	23.8 (19.2–26.4)	0.25 (0.23–0.27)
Dog	1539.0 (988.0–2066.0)	10.15 (6.62–11.97)	127.0 (96.0–170.0)	1.13 (0.81–1.64)
	2.0 (1.2–3.6)	0 (0– 0.03)	2.2 (1.8–3.0)	0.01 (0–0.01)
Rabbit	700.0 (531.0–848.0)	7.56 (5.63–9.29)	52.0 (46.0–59.0)	0.54 (0.48–0.64)
	3.6 (3.0–4.2)	0.04 (0.03–0.05)	52.6 (32.4–70.2)	0.53 (0.30–0.74)
Pig	455.0 (418.0–493.0)	6.79 (5.13–9.66)	33.0 (24.0–40.0)	0.10 (0.08–0.11)
	3.4 (2.4–5.4)	0.10 (0.07–0.12)	0	0
Man	163.0 (11.0–692.0)	5.51 (0.52–17.47)	16.0	0.12 (0.10–0.14)
	0.3 (0–1.2)	0.01 (0–0.04)	0.1 (0–0.3)	0
Monkey	952.0 (563.0–1341.0)	3.32 (1.73–4.90)	94.0 (75.0–113.0)	0.79 (0.58–0.93)
	2.1 (1.8–2.4)	0.01 (0–0.01)	2.7 (1.8–3.6)	0.01 (0–0.07)
Sheep	208.0 (227.0–397.0)	1.47 (0.97–1.80)	110.0	0.84
	14.8 (13.8–15.6)	0.12 (0.11–0.13)	3.6	0.02
Chicken	218.0 (170.0–295.0)	1.29 (0.94–1.62)	10.0 (5.0–16.0)	0.07 (0.04–0.10)
	1.8 (1.8–2.4)	0.01 (0.01–0.02)	10.0 (9.0–11.0)	0.13 (0.07–0.19)
Turkey	73.0 (63.0–83.0)	0.53 (0.52–0.54)	94.0 (69.0–113.0)	0.90 (0.62–1.10)
	7.2 (6.6–7.8)	0.05 (0.04–0.06)	2.7 (1.8–3.0)	0.02
	Brain		Ovary	
	Activity/g of tissue	Activity/mg of protein	Activity/g of tissue	Activity/mg of protein
Guinea pig	78.0 (74.0–87.0)	1.16 (1.01–1.32)	411.0 (355.0–471.0)	4.36 (3.76–5.48)
	7.2 (6.6–9.0)	0.10 (0.08–0.10)	32.2 (27.0–37.7)	0.36 (0.31–0.43)
Cat	72.0 (57.0–87.0)	6.26 (5.00–7.52)	51.0 (34.0–77.0)	4.71 (3.50–5.92)
	0.6 (0–1.2)	0.03 (0–0.06)	2.4 (1.2–3.6)	0.21
Ox	28.0 (11.0–31.0)	0.27 (0.22–0.32)	25.0 (15.0–35.0)	0.20 (0.10–0.50)
	1.6 (1.2–2.1)	0.02 (0–0.04)	1.7 (0.9–2.4)	0.02 (0.01–0.02)
Mouse	35.0 (33.0–38.0)	1.73 (1.30–2.41)	—	—
	8.8 (7.8–10.2)	0.33 (0.29–0.43)	—	—
Rat	176.0 (156.0–200.0)	5.92 (4.56–7.81)	87.0 (74.0–93.0)	2.17 (1.91–2.56)
	7.0 (6.0–8.4)	0.22 (0.17–0.25)	2.0 (1.8–2.4)	0.04 (0.03–0.06)
Dog	101.0 (94.0–105.0)	3.08 (2.69–3.64)	20.0 (12.0–28.0)	3.87 (2.69–5.05)
	3.7 (3.0–4.8)	0.14 (0.12–0.18)	0	0
Rabbit	86.0 (81.0–95.0)	1.34 (1.26–1.51)	17.0 (11.0–24.0)	0.33 (0.28–0.38)
	11.4 (10.8–12.6)	0.18 (0.16–0.23)	0	0
Pig	59.0 (42.0–80.0)	5.71 (4.66–6.75)	58.0 (41.0–76.0)	1.82 (1.68–1.95)
	9.3 (9.0–9.6)	0.31 (0.30–0.34)	0.6 (0–1.2)	0.01 (0–0.02)
Man	—	—	—	—
	—	—	—	—
Monkey	76.0 (53.0–98.0)	3.16 (2.57–3.78)	116.0 (110.0–126.0)	3.79 (2.88–4.70)
	5.4	0.26 (0.13–0.38)	0.9 (0–1.8)	0.02 (0–0.03)
Sheep	108.0 (86.0–123.0)	3.83 (3.04–4.97)	84.0 (77.0–92.0)	4.77 (4.47–5.06)
	4.2 (3.0–4.8)	0.16 (0.13–0.19)	1.6 (1.2–1.8)	0.05 (0.03–0.06)
Chicken	24.0 (17.0–31.0)	0.37 (0.28–0.47)	17.0 (15.0–18.0)	0.11 (0.10–0.12)
	6.0 (5.4–7.2)	0.20 (0.18–0.23)	65.1 (61.8–67.2)	0.42 (0.39–0.46)
Turkey	20.0 (10.0–32.0)	0.71 (0.50–0.99)	—	—
	2.7 (2.4–3.0)	0.08 (0.07–0.09)	—	—

Table 1—*continued*

Species	Spleen		Lung	
	Activity/g of tissue	Activity/mg of protein	Activity/g of tissue	Activity/mg of protein
Guinea pig	371.0 (267.0–485.0)	5.91 (5.77–6.05)	48.0 (43.0–53.0)	0.60 (0.47–0.71)
	17.3 (16.6–18.6)	0.18 (0.15–0.21)	14.7 (13.8–15.6)	0.19 (0.17–0.20)
Cat	69.0 (65.0–76.0)	1.02 (0.82–1.40)	78.0 (64.0–100.0)	1.97 (1.48–2.22)
	3.0 (2.4–3.6)	0.03 (0.02–0.04)	2.7 (1.8–3.6)	0.06 (0.04–0.07)
Ox	132.0	1.78	169.0	2.76
	3.6	0.04	4.2	0.06
Mouse	67.0 (65.0–68.0)	0.60 (0.58–0.62)	31.0 (30.0–33.0)	0.56 (0.48–0.65)
	8.2 (7.2–9.0)	0.16 (0.15–0.17)	1.6 (1.2–2.4)	0.04
Rat	194.0 (172.0–228.0)	2.50 (2.02–2.86)	166.0 (161.0–177.0)	3.07 (2.64–3.51)
	6.2 (6.0–6.6)	0.07 (0.06–0.07)	3.3 (2.4–4.2)	0.04
Dog	45.0 (27.0–56.0)	0.66 (0.58–0.73)	88.0 (57.0–117.0)	1.43 (0.90–2.51)
	0.7 (0–2.4)	0 (0–0.03)	2.0 (1.8–2.4)	0.01 (0–0.02)
Rabbit	61.0 (56.0–66.0)	0.72 (0.64–0.75)	69.0 (57.0–82.0)	0.79 (0.61–0.92)
	6.0 (5.4–6.6)	0.06 (0.05–0.07)	0.4 (0.3–0.5)	0.04 (0.03–0.05)
Pig	762.0 (567.0–957.0)	9.83 (7.66–12.00)	45.0 (38.0–55.0)	1.86 (1.46–2.50)
	6.3 (5.4–7.2)	0.08 (0.07–0.09)	1.8 (1.2–2.4)	0.03 (0.03–0.04)
Man	—	—	16.0	0.08
	—	—	0.9	0
Monkey	120.0 (110.0–139.0)	1.32 (1.01–1.56)	128.0 (96.0–157.0)	2.32 (1.57–3.30)
	5.0 (4.8–5.4)	0.05 (0.04–0.06)	1.0 (0–2.4)	0.01 (0–0.04)
Sheep	105.0 (93.0–120.0)	1.60 (1.29–2.10)	131.0 (109.0–142.0)	1.74 (1.37–2.16)
	4.9 (3.6–6.0)	0.08 (0.05–0.10)	7.2 (6.0–8.4)	0.08 (0.06–0.09)
Chicken	8.0 (6.0–9.0)	0.05 (0.04–0.06)	22.0 (15.0–28.0)	0.15 (0.12–0.20)
	27.1 (20.1–33.0)	0.21 (0.17–0.26)	5.4 (3.9–6.3)	0.03 (0.02–0.04)
Turkey	—	—	—	—
	—	—	—	—

Species	Testis		Kidney	
	Activity/g of tissue	Activity/mg of protein	Activity/g of tissue	Activity/mg of protein
Guinea pig	113.0 (98.0–124.0)	1.56 (1.41–1.76)	122.0 (108.0–132.0)	1.29 (1.14–1.44)
	15.0 (12.6–16.8)	0.20 (0.18–0.22)	13.0 (12.0–13.8)	0.14 (0.12–0.15)
Cat	59.0 (51.0–71.0)	1.32 (1.14–1.51)	14.0 (8.0–24.0)	0.15 (0.12–0.18)
	0	0	0.9 (0.6–1.2)	0 (0–0.01)
Ox	—	—	132.0	1.83
	—	—	20.4	0.28
Mouse	170.0 (143.0–209.0)	4.25 (4.07–4.46)	11.0 (10.0–14.0)	0.25 (0.17–0.35)
	8.4 (7.2–9.0)	0.19 (0.19–0.20)	10.2	0.24 (0.20–0.28)
Rat	—	—	117.0 (92.0–141.0)	1.98 (1.60–2.20)
	—	—	9.0 (8.4–9.6)	0.14 (0.13–0.15)
Dog	354.0 (352.0–357.0)	4.36 (3.55–5.96)	66.0 (54.0–82.0)	1.12 (0.84–1.48)
	0.2 (0–0.6)	0 (0–0.02)	3.1 (2.4–3.6)	0.03 (0.02–0.04)
Rabbit	76.0 (66.0–83.0)	1.02 (0.91–1.09)	59.0 (52.0–65.0)	0.68 (0.60–0.80)
	0.2 (0.1–0.3)	0.03 (0.02–0.03)	4.2 (3.6–5.4)	0.04 (0.03–0.05)
Pig	113.0 (65.0–161.0)	2.40 (1.26–3.53)	90.0 (88.0–93.0)	2.84 (2.57–3.10)
	3.9 (3.6–4.2)	0.07 (0.06–0.09)	3.9 (3.6–4.2)	0.09 (0.06–0.12)
Man	—	—	—	—
	—	—	—	—
Monkey	32.0	0.96	87.0 (77.0–100.0)	1.16 (1.25–1.78)
	1.2	0.03	11.1 (7.8–14.4)	0.10 (0.10–0.11)
Sheep	79.0 (76.0–86.0)	1.62 (1.34–1.88)	91.0 (81.0–101.0)	1.20 (1.12–1.33)
	21.8 (20.4–24.0)	0.44 (0.32–0.51)	3.9 (3.6–4.8)	0.05 (0.03–0.06)
Chicken	—	—	5.0 (3.0–7.0)	0.03 (0.02–0.05)
	—	—	37.0 (28.8–51.3)	0.26 (0.20–0.36)
Turkey	—	—	—	—
	—	—	—	—

vent our detection of the enzyme in some of the organ extracts reported here. Low-molecular-weight inhibitors might have been present in the crude homogenate used. Dialysis ought to be able to remove these. Inhibitors of high molecular weight might have been present, and these could either be non-enzymic or enzymic in character; both could be disclosed by mixing experiments, in which dialysed organ extracts of nearly zero synthetase activity are mixed with tumour extracts of known activity; dis-

proportionate decrements in the resultant activity could be ascribed to thermostable inhibitors. Enzymic inhibitors could be classed into four groups: enzymes consuming the substrate ATP, such as ATPase*; enzymes capable of consuming L-glutamine, such as L-glutaminase present in the kidney and brain, or of subtracting L-aspartate, such as the decarboxylases and transaminases. A careful balance sheet of the reaction will uncover such interfering enzymes. The identification of such factors as these has not, however, been practical in a survey of this type. However, enzymes hydrolysing the product L-asparagine would interfere most seriously, so that the activity of L-asparaginase in the samples was studied in every case.

Our observations show that the liver of the guinea pig and mouse showed the highest specific activity of L-asparaginase (95.8 μmol/h per g in guinea-pig liver; 100 μmol/h per g in mouse liver). Ohnuma *et al.* (1967) reported that guinea-pig liver contains L-asparaginase (2.8 units/g of liver; calculated as 168 μmol/h per g) in a higher concentration than the liver of the rat, chicken, mouse, rabbit, ox and pig. Tursky & Valovicova (1964) reported L-asparaginase activity in guinea-pig liver exceeding that found in the kidney or even serum and brain (380 μmol/h per g in the liver, 88.0 μmol/h per g in the kidney and 164.7 μmol/h per g in the serum).

Clementi (1922) was the first to observe the presence of L-asparaginase in several of the tissues of herbivorous animals. Geddes & Hunter (1928) later observed the presence of L-asparaginase in calf liver. Our studies show that ox liver contains low L-asparaginase activity compared with the liver of the other species studied (3.6 μmol/h per g). Steensholt (1944), who studied L-asparaginase activity in the liver of cold-blooded animals, observed that liver extracts of trout and plaice were capable of hydrolysing L-asparagine, but the liver of the frog was unable to do so. Milman *et al.* (1973) observed that many organs of the skate (*Raja ocellata*), including pancreas, contained L-asparaginase; however, the organs of the dogfish (*Squalus acanthias*) apparently were unable to hydrolyse L-asparagine. In the present study we observed the testis of most species to have approximately the same L-asparaginase activity as the ovary, whereas the kidney, spleen, brain and lung showed roughly comparable activities.

It appears that the pancreas, by virtue of its L-asparagine synthetase activity, may play an important regulatory role in the homeostasis of L-asparagine. This control may extend to the regulation of circulating concentrations of the amino acid, as Woods & Handschumacher (1971) have shown for the liver, or may be confined to the endogenous requirements of the pancreas. Cassano & Hansson (1965) reported that [^{14}C]glutamine, given intravenously, was rapidly taken up from the blood by several tissues of the mouse and that 5 min after administration the highest concentration was found in the pancreas. Further, the radioactivity was confined mainly to the exocrine part of the gland. Cassano & Hansson (1965) concluded that L-glutamine was most probably being used for the synthesis of pancreatic protein. For L-asparagine, the next lower homologue of L-glutamine, an analogous conclusion might be expected.

It is difficult to produce toxicity in the pancreas with L-asparaginase therapy in the mouse. This may be due to the high L-asparagine synthetase activity present in that organ. Comparatively, this activity is approximately equal to that found in the Leukaemia 5178Y resistant to L-asparaginase. In man, however, 30% of the patients receiving L-asparaginase in one study (Canellos *et al.*, 1971) showed some degree of pancreatic toxicity at post-mortem examination. The apparently low synthetase activity in human pancreas may not be sufficient to overcome the L-asparaginase-induced depletion of L-asparagine. The decrease of this amino acid in pancreas may play a significant role in the production of L-asparaginase toxicity.

Lastly, it should be pointed out that mouse pancreatic synthetase exhibits biochemical properties similar to those of mouse tumoural synthetase (Milman *et al.*, 1973). Both enzymes catalyse the formation of L-asparagine from L-aspartate+L-glutamine or NH_3 in the presence of ATP and Mg^{2+}: both exhibit product inhibition and chloride stimulation (Horowitz & Meister, 1972). Diet affects the pancreatic enzyme; a low-L-asparagine diet produces a significant increase in pancreatic synthetase activity in tumour-bearing mice, whereas tumoural synthetase is unaffected. It therefore appears that the pancreatic enzyme may be a counterpart of the tumoural enzyme.

This work is taken from a dissertation by Harry A. Milman to be presented to the Department of Pharmacology, The Graduate School of Arts and Sciences, The George Washington University, in partial fulfilment of the requirements for the Ph.D.

* Abbreviations: ATPase, adenosine triphosphatase.

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