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The Regulation of L-Asparaginase Activity in Rats and Mice

EFFECTS OF NORMAL AND MALIGNANT GROWTH, OF SEX AND OF DIETARY CHANGES

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1. The activity of L-asparaginase was very low in the liver of newborn rats and mice, and increased within a few days of birth. 2. In rats, but not in mice, the enzyme activity was higher in females than in males, was enhanced by administration of oestradiol, and was decreased by gonadectomy. 3. The enzyme activity decreased in mice starved or fed on a low-protein diet; in rats it was enhanced by starvation, by feeding them on a high-protein diet, or by administration of L-asparagine. 4. The asparaginase activity was decreased in regenerating liver, and was almost absent in the Morris hepatoma 5123.

L-Asparaginase (L-asparagine aminohydrolase, EC 3.5.1.1) has attracted considerable interest, since this enzyme has been used to cure leukaemias and lymphoid tumours in mice and rats (see review by Broome, 1968). L-Asparaginase activity is present in the liver of various animals (Ohnuma, Bergel & Bray, 1967) and in the serum of guinea pigs (Clementi, 1922) and of a few other species (Holmquist, 1963; Old, Boyse, Campbell & Daria, 1967). The aim of this investigation was to study the factors that may influence the activity of L-asparaginase in the liver of rats and mice.

The effects of age, of sex and sex hormones, of adrenalectomy and of cortisone, and of the dietary state were studied. Attempts to induce the enzyme were made by administration of asparagine and of aspartic acid. The activity of asparaginase in regenerating liver and in the Morris hepatoma 5123 was also investigated.

EXPERIMENTAL

Animals. Rats of the Wistar–Glaxo strain and mice of the Swiss strain were bred in this Institute and, except when stated otherwise, were fed on a standard complete laboratory diet supplied by Laboratorio D.ri Piccioni, Brescia, Italy. The 20% casein diet was as described by Bonetti, De Stefano & Stirpe, 1966; the 5% and the 50% casein diets were derived from the above, the variations of the casein content being compensated by appropriate changes of the sucrose and starch content. Partial hepatectomy was performed by the technique of Higgins & Anderson (1931) under ether anaesthesia. Sham-operated controls were laparatomized, the liver was exposed, and the abdomen sutured as for partial hepatectomy. The Morris hepatoma 5123 (Morris, Sidransky, Wagner & Dyer, 1960), transplanted in Buffalo rats, was from a strain given by Professor A. Fonnesu, Florence, Italy, and was obtained originally from the McArdle Memorial Laboratory, Madison, Wis., U.S.A. As far as possible, all treated animals were killed on the same day as their controls.

Determination of L-asparaginase activity. The method was derived from that of Ohnuma *et al.* (1967). The liver (or pool of livers in the case of small animals) or the hepatoma was homogenized in ice-cold 0·15 M-KCl (1 g. of tissue/19 ml. of KCl) in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 27000*g* for 15 min. at 0°, and 0·6 ml. of the supernatant was added to flasks containing 0·5 ml. of 40 mM-L-asparagine and 0·9 ml. of 0·1 M-borate buffer, pH 8·5, and incubated at 37° for 30 min. in air in a Dubnoff shaker. The reaction was stopped with 1 ml. of 10% (w/v) sodium tungstate and 1 ml. of 0·5 M-H_2SO_4, the samples were centrifuged, and the ammonia liberated was determined in samples (0·5 ml.) of the supernatant by the method of McCullough (1967). Values obtained from samples to which asparagine was added after the incubation were subtracted. With each experiment, a standard curve was obtained with $(NH_4)_2SO_4$ containing the same amount of asparagine as was present in the incubation mixture. Under these conditions, the amount of ammonia formed was proportional to the time of incubation and to the amount of liver extract used. The protein content of the liver extracts was determined by the method of Gornall, Bardawill & David (1949), or from the nitrogen content determined by a micro-Kjeldahl method. The latter procedure was used for samples that gave turbidity in the method of Gornall *et al.* (1949), as was the case with homogenates from regenerating and newborn liver.

RESULTS

Influence of age, sex, gonadectomy and hormones on L-asparaginase activity. The activity of L-asparaginase was very low in the livers of newborn rats and mice, and increased with age, more rapidly in rats than in mice (Table 1). In rats the activity per unit weight of liver or of liver protein seemed to increase

Table 1. *Activity of liver* L-*asparaginase in rats and mice of different age and sex*

The enzyme activity was determined as described in the Experimental section. Results are mean values ± S.E.M. The numbers of experiments are given in parentheses; the livers from several animals were pooled in each experiment with rats up to 4 days and with mice up to 10 days of age. M, male; F, female.

Animals, age and sex		L-Asparaginase activity (μmoles of NH_3/g. of liver/30min.)	(μmoles of NH_3/mg. of protein/30min.)	(μmoles of NH_3/100g. body wt./30min.)
Rats				
Less than 1 day	(7)	12·9± 3·8	0·20±0·05	—
2 days	(6)	37 ± 2·2	0·39±0·03	134± 8
4 days	(5)	108 ±11·3	1·18±0·08	344± 51
10 days, M	(6)	152 ±15·2	1·41±0·16	377± 40
26 days, M (Weaning)	(6)	207 ±17·9	1·69±0·15	839± 66
2 months, M	(6)	233 ±17·4	1·91±0·14	1145±109
2 months, F	(6)	409 ±22·5*	3·21±0·20*	1929±118*
3½ months, M	(6)	288 ±13·5	2·66±0·14	1309± 67
3½ months, F	(6)	515 ±14·8*	4·64±0·06*	1885± 82*
6 months, M	(6)	321 ±27·9	2·77±0·34	1137± 80
6 months, F	(4)	562 ±39·1*	5·21±0·73†	1954±224‡
Mice				
Less than 1 day	(6)	22 ± 2·4	0·31±0·04	—
10 days	(6)	51 ± 4·0	0·70±0·04	127± 9
24 days (Weaning)	(7)	163 ± 5·9	2·05±0·08	712± 45
2 months, M	(8)	437 ±20·7	3·55±0·13	2184±134
2 months, F	(8)	496 ±18·2	3·62±0·13	2492±117

* † ‡ Significantly different from males (*$P<0·001$, †$P<0·02$, ‡$P<0·01$).

for the entire age period considered (6 months), whereas the activity/100g. body wt. reached its maximum at 2 months and did not change afterwards.

The enzyme activity was higher in female than in male rats from 2 to 6 months of age, but no differences were seen between male and female mice (Table 1). This led us to investigate the effect of gonadectomy on L-asparaginase activity in the rat. The enzyme activity was lowered in ovariectomized females and became close to the values observed in intact males (Table 2). However, orchiectomy also lowered the asparaginase activity, and consequently a difference remained between ovariectomized and orchiectomized rats, which was significant and similar to the difference observed between intact female and male animals.

The administration of oestradiol brought about a significant increase (30%) in the asparaginase activity in the liver of male rats; whereas a less marked decrease (−15%) in the enzyme activity was observed in female rats treated with testosterone propionate. No changes were seen after adrenalectomy, and the administration of cortisone brought about only a small, although statistically significant, elevation of the asparaginase activity.

L-*Asparaginase activity in regenerating liver and in the Morris hepatoma* 5123. To ascertain whether the low activity of L-asparaginase observed in the liver of newborn animals was related to the higher mitotic activity, the enzyme activity was determined in the early phases of liver regeneration after partial hepatectomy. The activity was lower in partially hepatectomized rats, compared with their sham-operated controls (Table 3). The activity/100g. body wt. 24hr. after the operation was about 30% of that of controls, and only a slow increase was observed on the second and third days. The activity observed in sham-operated rats was higher than that in normal rats. This difference could be an effect of the operation, but the possibility of seasonal changes cannot be excluded, since these experiments were performed at a different time of the year.

The enzyme activity of the hepatoma 5123 was very low (about 13% of that of the liver). The asparaginase activity of the liver of tumour-bearing rats was comparable with that of normal animals.

Effect of dietary changes. In rats starvation caused initially a moderate decrease of L-asparaginase activity (Table 4), but after 3 days the activity was higher than in normal animals;

Table 2. *Effects of gonadectomy and adrenalectomy, and of the administration of testosterone, oestradiol, and cortisone on* L-*asparaginase activity of rat liver*

Experimental conditions were as in Table 1. Rats were gonadectomized at day 24, and killed when 2 months old. Testosterone propionate (Perandren; Ciba) (7 mg./rat/day) and α-oestradiol benzoate (Progynon; Schering) (100 μg./rat/day) dissolved in olive oil were injected subcutaneously for 7 days to 3½-month-old animals. Controls received an equal amount of olive oil. Adrenalectomy was performed 7 days before death. Cortisone (1 mg./rat/day) was injected intraperitoneally for 7 days as a suspension in 0·85% NaCl. Controls received an equal amount of 0·85% NaCl. M, male; F, female. The numbers of experiments are given in parentheses.

Sex	Treatment		L-Asparaginase activity (μmoles of NH_3/g. of liver/30 min.)	(μmoles of NH_3/mg. of protein/30 min.)	(μmoles of NH_3/100 g. body wt./30 min.)
M	None	(6)	215± 7·2	2·11±0·08	953± 51
M	Orchiectomy	(6)	169± 9·5*	1·56±0·10*	851± 45
F	None	(5)	406±32·1	3·74±0·28	1760±120
F	Ovariectomy	(5)	296±18·6†	2·44±0·14*	1502±139
M	Olive oil	(6)	299±12·1	2·95±0·09	1192± 64
M	Oestradiol	(6)	388±21·9*	3·81±0·17*	1678± 85*
F	Olive oil	(6)	547±11·9	4·54±0·10	2194± 97
F	Testosterone	(6)	461±24·6*	4·13±0·14	1800±108†
M	Sham-operation	(3)	462±12·6	3·94±0·28	1863± 82
M	Adrenalectomy	(3)	479±32·3	4·20±0·09	1717±100
M	NaCl	(6)	288±13·5	2·66±0·14	1309± 67
M	Cortisone	(9)	346±11·6*	3·18±0·14‡	1550± 44*
F	NaCl	(6)	515±14·8	4·64±0·06	1885± 82
F	Cortisone	(8)	586± 8·85	5·19±0·14†	2415± 55§

* † ‡ § Significantly different from controls (*$P<0·01$; † $P<0·02$; ‡ $P<0·05$; § $P<0·001$).

Table 3. L-*Asparaginase activity in regenerating liver and in the Morris hepatoma* 5123

Experimental conditions were as described in Table 1. Male rats 3½ months old were used in all experiments.

Animals		L-Asparaginase activity (μmoles of NH_3/g. of liver/30 min.)	(μmoles of NH_3/mg. of protein/30 min.)	(μmoles of NH_3/100 g. body wt./30 min.)
Sham-operated, 1 day	(4)	528±35·3	4·88±0·33	1914±113
Partially hepatectomized, 1 day	(4)	318±15·9*	2·94±0·17*	596± 44
Sham-operated, 2 days	(4)	476±24·0	4·51±0·08	2124± 80
Partially hepatectomized, 2 days	(4)	364±27·8†	3·08±0·28*	801±110
Sham-operated, 3 days	(4)	552±31·3	4·94±0·31	2213±139
Partially hepatectomized, 3 days	(4)	336±22·7*	3·14±0·29*	898± 70
Tumour-bearing rats				
Host liver	(3)	294±32·7	2·55±0·36	1072±109
Hepatoma	(3)	39± 7·2	0·34±0·03	—

* † Significantly different from sham-operated controls (* $P<0·01$, † $P<0·05$).

however, the activity/100 g. of initial body weight was similar to that of fed rats. The asparaginase activity was unchanged when the dietary protein was lowered to 5%, but was approximately doubled when it was raised to 50%. In mice, starvation caused a progressive decrease in asparaginase activity. A small but statistically significant decrease was caused also by the low-casein diet, whereas the 50% casein diet did not cause any change.

Effect of L-*asparagine and of* L-*aspartic acid.* Administration of L-asparagine to rats was followed

Table 4. *Effect of dietary changes on liver* L-*asparaginase activity*

Experimental conditions were as in Table 1. Animals were males, 2 months old at the beginning of dietary treatment. The experimental diets were given for 7–10 days. The numbers of experiments are given in parentheses.

Animals and treatment		L-Asparaginase activity (μmoles of NH_3/g. of liver/30 min.)	(μmoles of NH_3/mg. of protein/30 min.)	(μmoles of NH_3/100 g. of initial body wt./30 min.)	(μmoles of NH_3/100 g. of final body wt./30 min.)
Rats					
Normally fed	(5)	249 ± 14·5	2·46 ± 0·14	—	1208 ± 112
Starved for 1 day	(5)	260 ± 12·1	2·05 ± 0·10	909 ± 49	1046 ± 39
Starved for 3 days	(6)	443 ± 16·7*	3·99 ± 0·20*	1303 ± 52	1776 ± 63†
20% Casein diet	(8)	198 ± 16·8	1·90 ± 0·15		1026 ± 118
5% Casein diet	(7)	245 ± 30·1	2·12 ± 0·28		1062 ± 121
50% Casein diet	(8)	497 ± 23·2*	4·10 ± 0·17*		2836 ± 223*
Mice					
Normally fed	(4)	406 ± 13·7	3·20 ± 0·23		1987 ± 134
Starved for 1 day	(6)	301 ± 22·1	2·71 ± 0·23	1061 ± 166	1402 ± 225
Starved for 3 days	(6)	258 ± 22·1	2·20 ± 0·22‡	688 ± 119	1044 ± 146†
20% Casein diet	(8)	317 ± 23·6	2·59 ± 0·17		1865 ± 118
5% Casein diet	(8)	246 ± 13·1§	1·86 ± 0·14+		1503 ± 140
50% Casein diet	(8)	329 ± 17·3	2·55 ± 0·15		1743 ± 98

* † ‡ § Significantly different from controls (* $P < 0·001$; † $P < 0·01$; ‡ $P < 0·02$; § $P < 0·05$).

Table 5. *Activity of liver* L-*asparaginase after administration of* L-*asparagine and of* L-*aspartic acid.*

Experimental conditions were as in Table 1, and the animals were as in Table 3. L-Asparagine and L-aspartic acid (neutralized with NaOH) dissolved in 0·85% NaCl were injected intraperitoneally twice a day, each time at the dosage of 100 mg. (in 2 ml.) for rats and of 20 mg. (in 0·5 ml.) for mice. Controls received the same volumes of 0·85% NaCl. The numbers of experiments are given in parentheses.

Animals and treatment		L-Asparaginase activity (μmoles of NH_3/g. of liver/30 min.)	(μmoles of NH_3/mg. of protein/30 min.)	(μmoles of NH_3/100 g. of body wt./30 min.)
Rats				
NaCl for 8 days	(8)	282 ± 9·6	2·67 ± 0·07	1312 ± 57
L-Asparagine for 8 days	(5)	407 ± 27·2	3·75 ± 0·14*	1808 ± 107*
L-Aspartic acid for 8 days	(8)	304 ± 8·8	2·87 ± 0·10	1562 ± 59†
NaCl for 16 days	(8)	281 ± 7·2	2·82 ± 0·05	1356 ± 58
L-Asparagine for 16 days	(6)	423 ± 8·9*	3·98 ± 0·10*	1958 ± 93*
L-Aspartic acid for 16 days	(6)	333 ± 9·0*	3·12 ± 0·10†	1710 ± 82†
Mice				
NaCl for 8 days	(3)	344 ± 23·8	3·08 ± 0·28	1755 ± 107
L-Asparagine for 8 days	(7)	280 ± 9·0‡	2·67 ± 0·09	1276 ± 46‡
L-Aspartic acid for 8 days	(5)	273 ± 8·5	2·45 ± 0·10§	1654 ± 113
NaCl for 16 days	(4)	357 ± 19·8	3·55 ± 0·26	2115 ± 84
L-Asparagine for 16 days	(4)	290 ± 7·9†	2·90 ± 0·17	1400 ± 46*
L-Aspartic acid for 16 days	(7)	279 ± 6·2†	2·45 ± 0·03†	1589 ± 91†

* † ‡ § Significantly different from controls (* $P < 0·001$; † $P < 0·01$; ‡ $P < 0·02$; § $P < 0·05$).

by a slow increase in the activity of asparaginase, which after 16 days was 50% above the control value (Table 5). Administration of L-aspartic acid was practically without effect. In mice a small decrease in the enzyme activity followed the administration of asparagine or of aspartic acid.

DISCUSSION

The activity of L-asparaginase is very low in the liver of newborn rats and mice, and increases rapidly as the animals grow. Tower, Peters & Curtis (1963) observed a very low asparaginase activity in the serum of newborn and very young guinea pigs, and therefore it is possible that lack of asparaginase at birth is a common feature of several mammalian species. The activity of asparaginase is also very low in the Morris hepatoma 5123, and is decreased in regenerating liver. On the other hand, it has been reported that asparaginase has embryotoxic effects (Adamson & Fabro, 1968) and inhibits mitosis (Becker & Broome, 1967) and RNA polymerase activity (Becker & Broome, 1969) in regenerating liver. It is tempting to correlate these observations, as well as the anti-tumour activity of asparaginase, and to postulate a negative correlation between the activity of this enzyme and tissue growth.

Sex is another physiological factor affecting L-asparaginase. This seems peculiar to rats, since no difference related to sex was observed in the enzyme activity in mouse liver or in guinea-pig serum (Tower *et al.* 1963). The higher activity observed in the liver of female rats may be caused by the female hormones, as is shown by the effects of ovariectomy and of administration of oestradiol.

The influence of dietary changes was also different in rats and mice. In mice, the enzyme activity decreased moderately during starvation and protein deficiency. In rats, an increase in asparaginase activity was observed after 3 days of starvation, or with a high-protein diet. Another difference between rats and mice is the response of the enzyme to asparagine and to aspartic acid. Administration of both substances caused a slight decrease in asparaginase activity in mice, whereas asparagine and to a lesser extent aspartic acid administration increased the enzyme activity in rat liver. Thus it seems that the asparaginase activity of rat liver is regulated at least in part by the substrate load, and by the amount of protein (asparagine?) in the diet. This might explain the rise in asparaginase activity that occurs in rats after a period of food deprivation, i.e. when glycogen is exhausted and fat depots are diminished and the animals survive by consuming their own protein. However, the total amount of enzyme, as estimated by the activity/100g. initial body wt., is not significantly increased in starved rats. This indicates that the rise in the asparaginase activity per unit weight of liver or per unit of liver nitrogen is not due to increased synthesis of new enzyme, but probably to preferential maintenance of asparaginase during starvation, as has been observed with arginase in the rat (Schimke, Brown & Smallman, 1963) and with xanthine oxidase in the chick (Stirpe & Della Corte, 1965).

Note added in proof. After this paper had been submitted for publication, we were able to read a report by Patterson & Orr (1969). These investigators observed that the L-asparaginase activity of rat liver is: (i) lower in foetuses and young rats as compared with adult animals; (ii) decreased during liver regeneration; (iii) slightly decreased in animals fed on asparagine-free diet; (iv) lowered in animals bearing Walker carcinoma, but unchanged in the liver of animals bearing other tumours.

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