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A Study of the *in Vitro* Effect of L-Asparaginase, EC-1 and EC-2, on the Glycine and Nucleic Acid Contents in Regenerating Liver of Albino Rats

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L-Asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1.) is known to be present in many animal tissues, bacteria and plants. It is also known to catalyze the irreversible deamidation of L-asparagine producing L-aspartic acid and ammonia. It is now well established as the antitumor agent in guinea pig serum (Kidd, 1953a), of which activity has been demonstrated with varieties of leukemias in mice (Boyse *et al.*, 1963: Mashburn and Wriston, 1964) as well as the Murphy-Sturm lymphosarcoma (Ainis *et al.*, 1958: Kidd, 1953b) and the Walker-256 carcinosarcoma (Kwak *et al.*, 1961) in rats.

L-Asparaginase obtained from yeast (Broome, 1965) and *B. coagulans* (Manning and Campbell, 1957), however, was shown to display no such antitumor activity. In addition to its antitumor activity, the L-asparaginase prepared from *E. coli* also inhibits the phytohaemagglutinin-induced blastogenesis of human lymphocytes (Astaldi *et al.*, 1969: Weiner *et al.*, 1969). Several evidences have been presented for the inhibition which showed (1) that the enzymatic degradation of L-asparaginase depletes L-asparagine which is essential for transformation (Prager and Derr, 1971); (2) that the inhibitory mechanism may be more complex than depletion of the amino acid and instead an operation of the inhibition of the glycoprotein synthesis might be involved (Prager and Derr, 1971); and (3) that the inhibitory effects could be due to interference with the binding of antigen to the surface of the lymphocyte (Fidler *et al.*, 1971).

Studying the L-asparaginase, Campbell *et al.* (1967) observed two activities of the enzyme in *E. coli* B and designated EC-1 and EC-2 with the evidence that their EC-2 was a potent antilymphoma agent, of which physical properties were recently studied extensively by Cammack *et al.* (1972). The existence in *E. coli* of two forms of L-asparaginase is also confirmed so far by Laboureur *et al.* (1971a) and Rauenbusch *et al.* (1971), the latter group designating the two forms as L-asparaginase A and B. Plaquet *et al.* (1971) also reported their isolation of two distinct L-asparaginase activities from guinea pig liver, by means of centrifugation of homogenates, precipitation with Na_2SO_4, DEAE-cellulose chromatography, and gel filtration on Sepha-

dex G-200. Moreover, the subunit structure of the enzyme has also been elucidated recently by several authors (Mashburn *et al.*, 1971: Laboureur *et al.*, 1971b: Tosa *et al.*, 1973) through the dissociation experiments in the presence of 3*M* guanidine-HCl, 0.1% sodium dodecyl sulfate, and of 7*M* urea.

From these studies, it became quite apparent that the two isoenzymes differ from each other by several criteria: solubility in ammonium sulfate solution, chromatographic behaviour, enzyme activity as a function of pH, antitumor activity (Campbell *et al.*, 1967), intracellular localization in anaerobically grown *E. coli* (Cedar and Schwartz, 1967), amino acid composition and amide content as well as isoelectric point, though neither optical rotatory dispersion nor circular dichroism data provided evidence for significant differences in the peptide chains of the two forms (Laboureur *et al.*, 1971a).

There are further evidences that after disagregation of the enzyme by isoelectric focussing in 6*M* urea, the subunits formed were shown to retain enzymic activity but to differ in stability and that the electrofocussing pattern results from different aggregations of these subunits (Mashburn *et al.*, 1971: Laboureur *et al.*, (1971b).

It is, however, a little premature to ascribe the antitumor activity of EC-2 asparaginase to the various differences cited above and to some speculations with regard to the mechanism of its antitumor activity (Broome, 1968: Ryan, and Sornson, 1970: Maral, 1971). But, in addition, it has recently been demonstrated that this enzyme delays the mitotic activity which occurs in the residual liver of rats approximately 25 to 35 hrs. after 70% hepatectomy (Becker and Broome, 1967). According to Becker *et al.* (1970), a single dose of 600 units of the enzyme at the time of operation has been demon strated to delay synthesis of DNA for an interval of approximately 10 hrs. It was pointed out that the results obtained with the regenerating rat liver differs from the permanent inhibition of growth which asparaginase causes in various tumors (Becker and Broome, 1967).

After removal of a considerable amount of tissue from liver in the normal rat, initiation of an extremely rapid process of growth is followed by the restoration of the lost liver mass, the sequence of events resembling the rapid growth of tumor mass. The present paper was designed, therefore, with a purpose to determine whether or not in the L-asparaginase inhibition of mitosis in regenerating liver, the mitotic block occurs only with EC-2, as reported in the enzyme inhibition of growth of certain tumors (Campbell *et al.*, 1967), with affirmative results.

MATERIALS AND METHODS

Bacterial strain. *E. coli* 0112a was obtained from the Department of Microbiology, School of Medicine, Seoul National University, through the courtesy of Assoc. Prof. W. H. Chang, and the cultures were maintained on slopes of nutrient agar, allowing to grow for 2 to 3 days at 22°C and storing at 4°C.

Culture media and harvesting of cells. A culture media, containing 0.15% peptone, 0.5 % beef extract, 0.5% KH_2PO_4 and 0.1% L-asparagine, was used, adjusting its pH to 7.0 before sterilization at 121°C of pressure for 15 min.

To inoculate the inoculum medium, a 2- to 3- day-old growth on nutrient agar slants was used, providing the final inoculum with the growth for 24 hr. in the appropriate liquid medium; and a 1:9 (v/w) ratio of final

inoculum to growth medium was used throughout the experiment. In order to have shaken cultures, 500ml of the medium was placed in 1-liter conical flasks which were inoculated and held at 35°C for 24 hrs. Production of L-asparaginase was tested with the use of shaken cultures at 22°C throughout.

At the end of the growth period, the cells were centrifuged at 23,000×g for 30 min. at 4°C, washed twice with 0.25*M* sucrose solution, and resuspended in 0.05*M* Tris buffer, pH 8.4, making the final concentration of 20%, for the enzyme assay.

Protein determination. Protein concentration during the purification procedure was monitored with the use of the Folin's phenol reagent (Lowry *et al.*, 1951), adopting the modified procedures reported by Oyama and Eagle (1956).

Enzyme assay. Assay of the L-asparaginase was carried out at 37°C after Meister's procedure (1955) with some modification as follows: the standard assay mixture contained, in a total volume of 2.7ml, 20 μmoles of 0.04*M* L-asparaginase and 200 μmoles of 0.1*M* sodium borate buffer, pH 8.4. For the determination of EC-1 and EC-2 in the presence of one another, a second assay was made using 200 μmoles of 0.1*M* acetate buffer, pH 5.0, in the place of the sodium borate buffer, and calculated their respective values of activities following the experimental equation reported by Campbell *et al.* (1967).

Assay mixtures were incubated at 37°C for exactly 30min., followed by addition of 0.4 ml. of 2/3 *N* sulfuric acid and 0.2ml. of 10% sodium tungstate solution. The incubation mixtures were mixed thoroughly and centrifuged. Adding 2ml. of phenol color reagent and 2ml. of alkaline hypochlorite solution to 2.0ml. of the clear supernatant solution after the method of Caraway (1966), the reaction mixture was kept in a water bath at 37°C for 15min. to develop color due to the liberation of ammonia. Colorimetry was performed with the Spectronic 20 at 630*nm*. One unit of enzyme activity was defined as one μ mole of ammonia liberated during 30min. incubation at 37°C.

Fractionation and partial purification of EC-1 and EC-2. After the fractionation procedure reported by Mashburn and Wriston (1966), a sample of *E. coli* was suspended in 0.05 *M* Tris buffer, pH 8.4, to have 20% (w/v) suspension and subjected to repeated sonication (30 sec. at a time for 20 min.) with a sonifier (Branson Sonic Power Co.). The cell free extracts were treated to remove nucleic acids by the dropwise addition of 0.05 volume of 1.0*M* $MnCl_2$ solution with constant stirring.

After stirring the solution for 45min., it was centrifuged at 23,000×g for 45min. and the residue was discarded, the supernatant of which was treated to remove polysaccharides by the addition of 0.06 volume of 1.0 *M* $BaCl_2$ solution with constant stirring for 20 min. After removal of the precipitated material by centrifugation, the supernatant was saturated to 2.0 *M* with ammonium sulfate.

Adjusting the pH of the suspension to 8.0, the resulting precipitate (As-1) was collected by centrifugation at 13,000×g for 20 min. and redissolved by dialysis against the distilled water and against 0.02 *M* phosphate buffer, pH 8.0. The supernatant was again brought to be saturated to 4.0 *M* with ammonium sulfate; and adjusting the pH again to 8.0 the precipitate (As-2) was collected and treated as with the As-1.

Both L-asparaginase fractions were further

purified by DEAE-cellulose chromatography. The DEAE-cellulose was pre-equilibrated with 0.02 *M* sodium phosphate buffer, pH 8.0, and the continuous sodium chloride gradient was applied by the addition to the mixing chamber of 0.35 *M* sodium chloride in 0.02*M* sodium phosphate buffer, pH 8.0. Estimating the protein contents and enzyme activities, elution profiles of the enzyme in both fractions were obtained, followed by salting-out the As-1 fraction with 2.0 *M* ammonium sulfate and the As-2 fraction with 4.0*M* ammonium sulfate. The former (EC-1) and the latter precipitates (EC-2) were collected by centrifugation at 13,000×g for 20 min. and redissolved by dialyzing against distilled water and 0.02*M* phosphate buffer, pH 8.0, respectively.

Partial hepatectomy and preparation of the regenerating liver tissue. Fifty percent partial hepatectomy was performed on 8 female Sprague-Dawley albino rats (200±10g), removing the median and left lobes, along with 8 sham operations.

Half of the total experimental animals were sacrificed 24 hr. after operation and the rest 48 hr. after operation, and samples of the regenerating livers were collected, pooled and homogenized (10%, w/v) in distilled water.

For *in vitro* incubation 6.0ml. of each enzyme preparation was added to 15.0 ml. of tissue samples, and the mixture was incubated at 37°C for 30 min. The reaction was terminated by the addition of 5.0ml. of 20% $HClO_4$ and the mixtures were centrifuged at 600×g for 30min. The resulting supernatant was used for the glycine and the precipitate for the nucleic acid determination respectively.

Glycine determination. Determination of the glycine content in the supernatant was performed after the method of Alexander *et al.*, (1945) as modified by Christenson *et al.*, (1951), using the color reaction of glycine (Eegriwe, 1937).

Extraction and determination of the nucleic acids. Samples were twice precipitated with 20% $HClO_4$ in an ice bath to remove the acid-soluble fraction, with the subsequent delipidation of the samples with the Bloor reagent. The residue was washed twice with ether, air-dried and ground into powder.

Both DNA and RNA were extracted from these lipid-free powders of tissues after the method of Schmidt and Thannhauser (1945), and color reactions were performed by the use of the orcinol reaction for RNA and diphenylamine reaction for DNA as reported by Dische (1930, 1937). For the reference standard, calf thymus DNA and yeast RNA (Fisher products) were used and all the colorimetry was carried out by the Beckman DU-Spectrophotometer.

RESULTS

I. Partial purification of L-asparaginases, EC-1 and EC-2

Attempt of partial purification of the L-asparaginases, EC-1 and EC-2, by using a combination of ammonium sulfate and DEAE-cellulose column chromatography, after removal of nucleic acids with $MnCl_2$ precipitation, resulted in more than 70-fold purifications as tabulated in Table I and II.

As pointed out elsewhere (Campbell *et al.*, 1967), the elution profile of the enzyme activities from the DEAE-cellulose column showed two distinctively different peaks, when the enzyme was eluted with sodium chloride. The first peak represents EC-2 (Fig. 2) and the second EC-1 activities (Fig. 1). It was quite apparent that most of the protein of As-1 fraction having no L-asparaginase activity was eluted in the vicinity of EC-2, while the protein of As-2 fraction was eluted in the

Table I. Summary of partial purification of the L-asparaginase, EC-1 from *E. coli*.

purification steps	Total Activity (u)	Total protein (mg)	Specific Activity (u/mg. protein)	yield (%)	purification fold
Cell free extract (sonicate)	3.07	82,830	37.1×10^{-6}	100.0	1
AS-1 fraction	2.49	936	26.6×10^{-4}	81.1	72
AS-2 fraction	—	811	—	—	—
DEAE-cellulose column eluate	0.66	209	31.6×10^{-4}	21.5	85

Table II. Summary of partial purification of the L-asparaginase, EC-2 from *E. coli*.

purification steps	Total Activity (u)	Total protein (mg)	Specific Activity (u/mg. protein)	yield (%)	purification fold
Cell free extract (sonicate)	3.44	82,830	41.5×10^{-6}	100.0	1
AS-1 fraction	—	936	—	—	—
AS-2 fraction	1.82	811	22.4×10^{-4}	52.9	54
DEAE-cellulose column eluate	1.26	405	31.1×10^{-4}	36.6	75

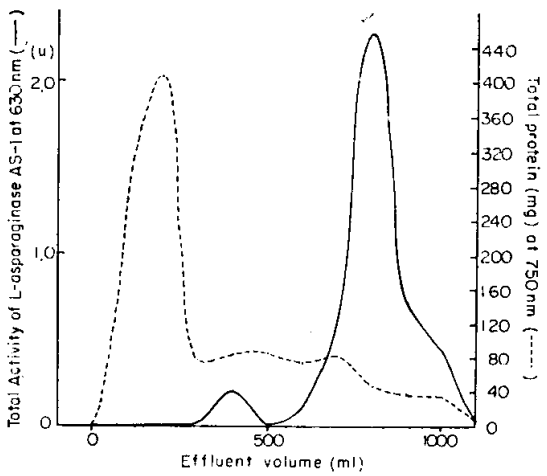

Fig. 1. Elution profile of DEAE-cellulose column chromatography of the As-1 fraction obtained from *Escherichia coli*.

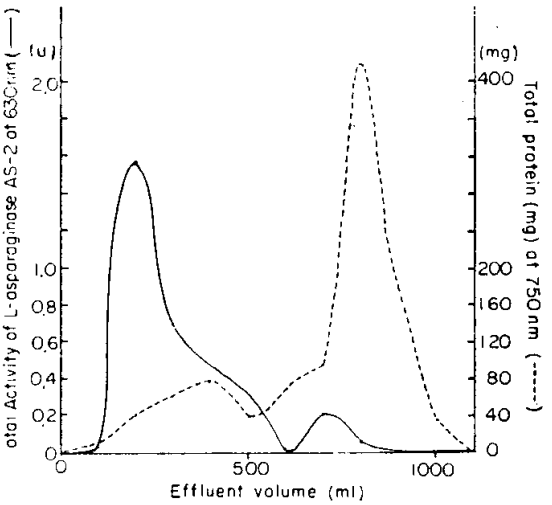

Fig. 2. Elution profile of DEAE-cellulose column chromatography of the As-2 fraction obtained from *E. coli*.

vicinity of EC-1.

And it seems to be safe to say, from Fig. 1 and 2, that the separation of EC-1 and EC-2 was quite satisfactory in the present study and that the protein contents in both fractions were so close that the specific activities of both enzymes were also similar, 26.6×10^{-4} units/mg. protein for EC-1 and 22.4×10^{-4} units/mg. protein for EC-2 (Table I and II).

II. Comparison of V_{max} and K_M values of EC-1 and EC-2.

Lineweaver-Burk plots of EC-1 and EC-2 of

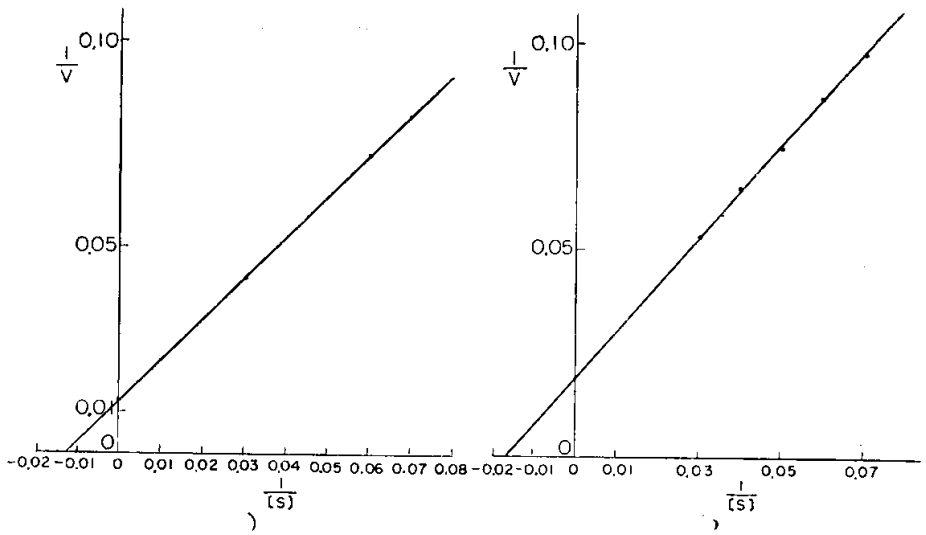

Fig. 3. Lineweaver-Burk plots of L-asparaginase, EC-1 (a) and EC-2(b), purified partially from *E. coli*.

the present preparations are illustrated in Fig. 3. From the linear forms K_M and V_{max} of the EC-1 were derived to be 57.14 μmoles and 51.28 μM/min. and those of EC-2 to be 74.62 μmoles and 76.92 μM/min., the former being lower than the latter.

III. In vitro Effects of L-asparaginase, EC-1 and EC-2 upon regenerating liver tissue.

It was reported that a measurement of all of the amino acids of normal tissues, of susceptible tumors, and of resistant tumors after injection of *E. coli* L-asparaginase indicated that there was only one amino acid change which was unique to the susceptible tumor. Glycine decreases in the susceptible tumor but not in the resistant tumor or the normal tissues (Ryan and Sornson, 1970). If it is the real picture, EC-2 may be expected to exert such an effect while EC-1 does not in susceptible tumor tissues, and the effect was compared in regenerating liver tissue *in vitro*, together with the effect on the DNA and RNA contents in the tissue, since it was reported that the enzyme affects mitotic activity in regenerating tissue (Campbell *et al.*, 1967). Table III shows the comparison with regard to the glycine and nucleic acid contents in regenerating liver tissue as affected *in vitro* by L-asparaginase EC-1 and EC-2 obtained from *E. coli*.

As is apparent from Table III, the glycine contents of the livers of sham operated rats did not show significant variability, as compared to the control value of 2.027μg. glycine-N/10ml. distillate, between the two groups, one analyzed after 24 hr. and another 48 hr. after sham operation.

In regenerating liver tissue, however, the glycine content increased up to 4.959μg. glycine-N/10 ml. distillate from the control value at 24 hr. after partial hepatectomy and resumed control level at 48 hr. after operation (2.493μg. glycine-N/10ml. distillate), without addition of EC-1 or EC-2.

An addition of EC-1 brought no significant variation to any group, while the addition of EC-2 to the regenerating liver tissue at 24hr. after partial hepatectomy resulted in a drastic decrease in its glycine content, from 4.959μg. glycine-N/10ml. distillate (without enzyme)

Table Ⅱ. Comparison of the *in vitro* effects of L-asparaginase EC-1 and EC-2 obtained from *E. coli* on the glycine and nucleic acid contents in the regenerating liver tissue of albino rats.

Sacrificed time	Animals (the number of rats)	Enzyme added	μg. Glycine-N per 10 ml. distillate	μg. DNA per 100 mg. delipidated dry powder	μg. RNA per 100mg. delipidated dry powder
0 hr.	Control (2)	none	2.027	90.36	11.23
24hr. after operation	sham operated (4)	none	2.192	103.61	12.77
		EC-1	2.192	96.39	13.16
		EC-2	2.110	91.57	13.55
	50% hepatectomized (4)	none	4.959	151.81	33.10
		EC-1	5.068	163.86	32.32
		EC-2	2.658	134.94	13.74
48hr. after operation	sham operated (4)	none	2.137	106.02	13.35
		EC-1	2.164	139.76	13.55
		EC-2	2.137	137.35	12.19
	50% hepatectomized (4)	none	2.493	118.07	13.94
		EC-1	2.795	132.53	14.52
		EC-2	2.603	110.84	13.16

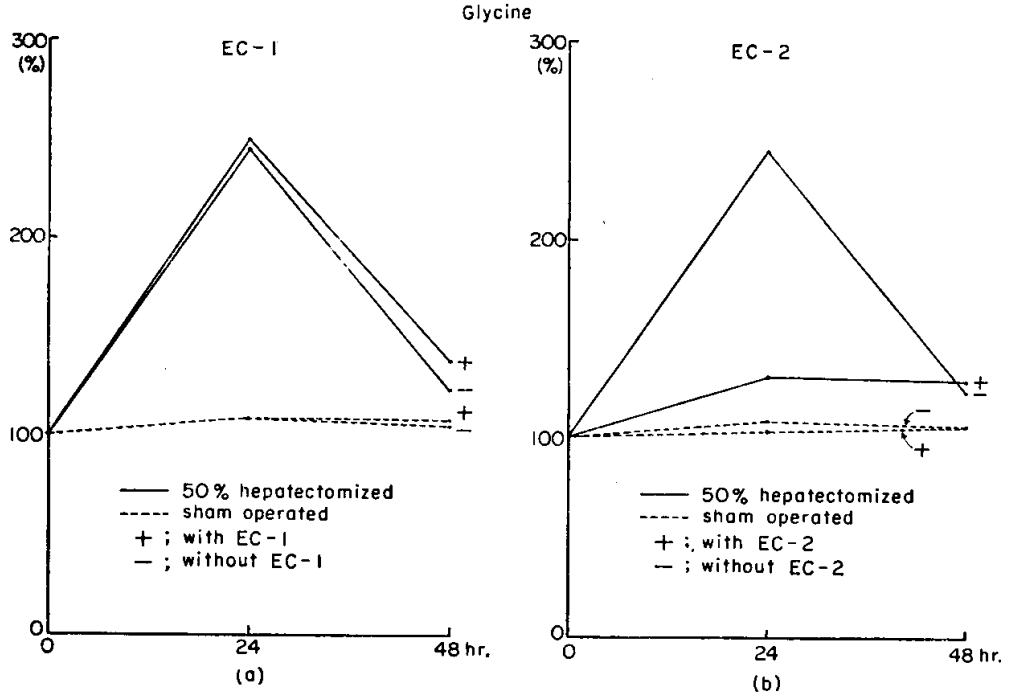

Fig. 4. Effects of L-Asparaginase, EC-1 (a) and EC-2 (b) on the variation of glycine contents in the partially hepatectomized and sham-operated rat liver homogenates.

to 2.658 μg. glycine-N/10 ml. distillate (with EC-2), the effect of which was not apparently observed at 48 hr. after partial hepatectomy. In other words, the glycine content in the liver of sham operated animal showed no fluctuation to be of note, but that in the regene-

rating liver of the animal rose abruptly during the first 24 hr., when actual regeneration process was under the way with an increment of as much as approximately 2.5-fold (Fig. 4, a and b). It was, however, very interesting to note that the EC-1 had actually no effect on this tendency, but that EC-2 seemed to suppress the increment and abolished the characteristic rise in the glycine content in the regenerating liver tissue (Fig. 4, b).

As for the DNA content, active synthesis of DNA seemed to take place right after partial hepatectomy and reaching to its peak value during the first 24 hrs. (from 90.36 to 151.81 μg./100mg. dry delipidated powder) with subsequent decline at the end of 48hr. after the operation (106.02 μg./100mg. dry delipidated powder) (Table III). Addition of EC-1 to the regenerating liver tissue showed almost the same tendency as displayed by EC-2 toward the glycine content in the same tissue as described above; that is, no significant effect among the groups observed.

The addition of EC-2, however, to the regenerating liver tissue at 24 hr. after partial hepatectomy depressed the increase of the DNA content in the tissue (134.94μg./100mg. dry delipidated powder), which otherwise would have increased from the control value of 90.36 to 151.81 μg./100mg. dry delipidated powder (Fig. 5, a and b).

Variations in the RNA contents in the liver tissues as affected by the EC-1 and EC-2 was as close and apparent as that observed with the glycine content (Table III, Fig. 6, a and b). It was a unique characteristic to note that the RNA content in the regenerating liver tissue was rising eminently during the first 24hr. after partial hepatectomy to almost a three-fold increase, from 12.77 to 33.10μg./

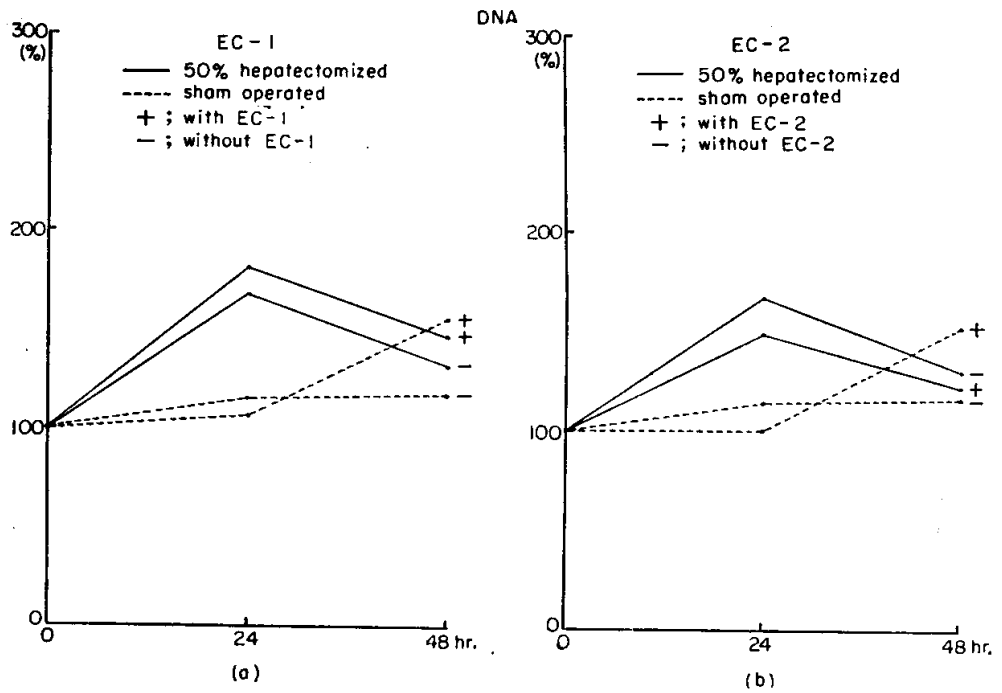

Fig. 5. Effects of L-Asparaginase, EC-1 (a) and EC-2 (b) on the variation of DNA contents in the partially hepatectomized and sham-operated rat liver homogenates.

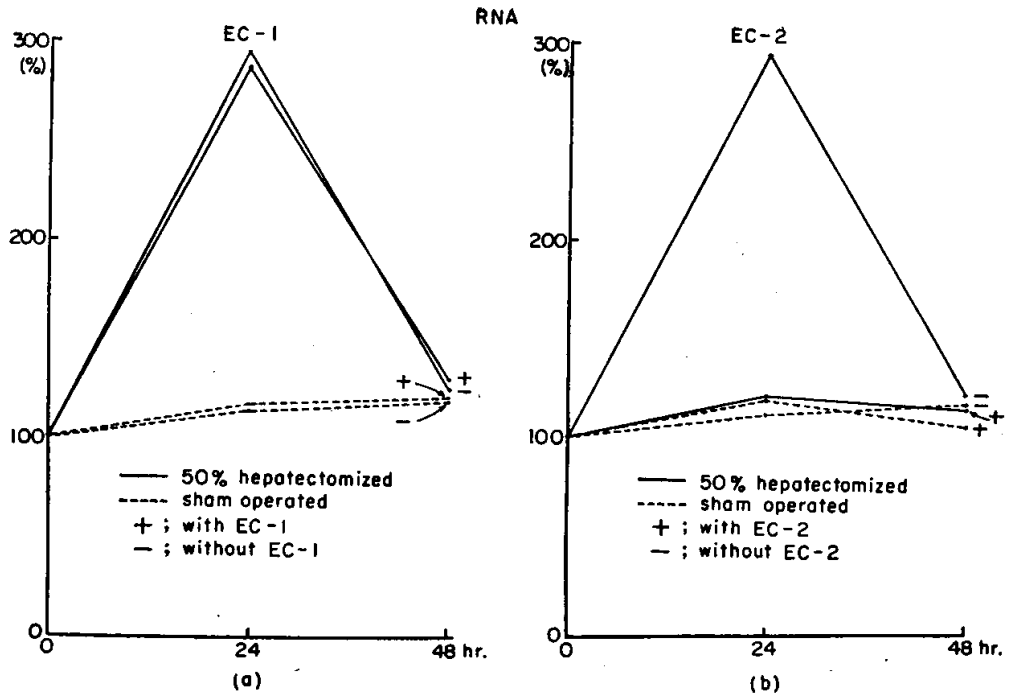

Fig. 6. Effects of L-Asparaginase, EC-1 (a) and EC-2 (b) on the variation of RNA contents in the partially hepatectomized and sham-operated rat liver homogenates.

100mg. dry delipidated powder (Table III), which was resuming its original value at the end of 48hr. after operation (13.94μg./100mg. dry delipidated powder).

It was also following a similar pattern of change in the RNA content in the regenerating liver tissue after addition of L-asparaginase EC-1 and EC-2, comparable as that observed with regard to the variation in the glycine content, EC-1 displaying no significant effect. But EC-2, on the other hand, abolished the almost a three fold increase in the RNA content in the regenerating liver tissue at 24 hr. after operation (Fig. 6, b).

It has been generally accepted that, in the regenerating liver tissue, a wave of mitosis occurs between 25 and 36 hrs. after hepatectomy following stages of RNA, protein and DNA synthesis. In our experiment, a three-fold rise in the RNA content in the tissue was observed with the decline after at 24 hrs. after operation with the subsequent decline. It was also observed that EC-2 inhibited the sharp increment in RNA content in early regeneration period.

DISCUSSION

Although it was suggested by Becker *et al.* (1970) that the administration of L-asparaginase delays the mitotic response significantly in the residual liver after partial hepatectomy, it was quite evident, from the present paper, that the effect could be brought only by the L-asparaginase EC-2. Its addition to the regenerating liver tissue resulted *in vitro* in a remarkable decrease not only in RNA but also in the glycine contents of the tissue, while L-asparaginase EC-1 showed no effect.

With regard to these two L-asparaginases,

the difference in its solubility in ammonium sulfate and in chromatography with DEAE-cellulose was well confirmed in the present study as reported by Campbell *et al.* (1967) together with other differences in properties (Greenstein and Price, 1949: Campbell *et al.*, 1967: Boyse *et al.*, 1967: Mashburn *et al.*, 1967). It has hardly been known, however, that only EC-2 L-asparaginase is the potent mitotic inhibitor as is apparent from our results, although there have been accumulated data dealing with the subunits and isoenzymic forms of the L-asparaginase recently (Laboureur *et al.*, 1971 a: Plaquet *et al.*, 1971: Laboureur *et al.*, 1971 b).

Ryan and Sornson (1970) reported that administration of either *E. coli* asparaginase or guinea pig serum to C3H/HE mice with the 6C3HED lymphosarcoma is followed by depression of glycine in the tumor. They have shown that, under appropriate conditions, it is possible to completely inhibit the effects of asparaginase by an injection of asparagine and that glycine also antagonizes the effects of the enzyme.

The present data also suggest that glycine plays an important role in asparaginase inhibition of mitosis as in the growth of lymphomas, presumably because of the fact that actively growing tissue, such as regenerating and tumor tissues, synthesizes cellular glycine from glyoxylic acid and asparagine as has been pointed out by Meister *et al.* (1952). The depletion of asparagine, therefore, by means of asparaginase would result in the deprivation of glycine in the regenerating tissue. Little or no effect on the sham operated control intact liver is observed because the usual conversion of serine to glycine may be operating to overcome the depletion of glycine.

Delayed and/or lowered synthesis of DNA was also apparent as reported by Becker *et al.* (1970), but its the magnitude was far lower than the remarkable decrease observed in the RNA content, which was again, in the present paper, a unique characteristic of the result brought about only by the L-asparaginase EC-2. Therefore, only the EC-2 enzyme can produce the glycine depletion, lowering efficiency in purine biosynthesis in the regenerating liver tissue.

Another important or perhaps a unique mechanism, which lowers the RNA content in the regenerating liver tissue, may be the involvement of RNA polymerase, which is reportedly inhibited by L-asparaginase (Becker and Broome, 1969). L-asparaginase EC-2, therefore, seems to inhibit the RNA polymerase activity in the regenerating tissue, thus suppressing RNA biosynthesis.

Although the foregoing discussion would be no more than a speculation with regard to the mechanism operated by the EC-2 asparaginase, obviously important fact still remains to be studied further a fact that only L-asparaginase EC-2 can produce the mitotic inhibition, while EC-1 can not. Micu *et al.* (1971) recently reported that rabbits given L-asparaginase for 1-2 weeks showed a decrease in the number of splenic pyroninophilic and mono-, bi-, and polynucleated lymphocytes, and a decrease in lymphocyte RNA synthesis, whose evidence lacked the selective inhibitory mechanism pertaining to the L-asparaginase EC-2 for the phenomena.

According to the extensive study by Cammack *et al.* (1972) and Campbell *et al.* (1967), it is quite clear that two L-asparaginases, EC-1 and EC-2 obtained from *E. coli*, together with that obtained from *Erwinia carotovora*, are different molecular species catalyzing the same reaction; but they could still

provide no evidence for the mitotic inhibition displayed by only EC-2 in the regenerating liver tissue as is evident from the present study, which seems to await further elucidation.

However, the mitotic arrest displayed *in vitro* of the present study by the L-asparaginase EC-2 appeared not to be permanent, unlike the case reported to be permanent in lymphomas, and coincide with the *in vivo* observation reported by Becker and Broome (1967). It was only eminent during the first 24hr. after partial hepatectomy, which resumed to the original values with regard to the glycine and the nucleic acid contents.

CONCLUSION

Two L-asparaginases, designated EC-1 and EC-2, have been partially purified from *E. coli* 0112a, and incubated respectively with the homogenates of the regenerating liver tissues of albino rats at 24 and 48hr. after partial hepatectomy, with the following conclusion.

(1) EC-2 exerts *in vitro* an effect to lower the nucleic acid contents in the regenerating liver homogenate, while EC-1 does not.

(2) EC-2 also lowers the content of glycine in the regenerating tissue homogenate, while EC-1 does not.

With the above results, possible mechanisms were discussed regarding the mitotic arrest selectively displayed by EC-2.

—국문초록—

흰쥐 再生肝의 glycine 및 核酸含量에 미치는 L-asparaginase, EC-1 및 EC-2의 影響에 관한 硏究

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조 경 혜

E. coli 0112a 菌株에서 EC-1 및 EC-2 兩 L-asparaginase를 部分精製하고, 이를 肝部分切除를 施術한 흰쥐에서 施術後 24, 48 時間만에 얻어진 再生肝組織과 함께 각기 incubate하여서 다음과 같은 結論을 얻었다.

(1) EC-2는 흰쥐·再生肝均等液의 核酸含量을 減少시키지만 EC-1은 아무런 影響도 미치지 않는다.

(2) EC-2는 역시 흰쥐再生肝均等液의 glycine 含量의 減少를 가져오지만 EC-1은 그렇지 아니하다.

이상의 結果로써 EC-2에 의해서만 mitosis가 抑制되는 機轉을 考察하였다.

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