The inhibition of lymphocyte mitogenesis by asparaginase: a still unexplained phenomenon

by D. Kafkewitz* and A. Bendich

Department of Zoology and Physiology, Rutgers University, Newark (New Jersey 07102, USA) and Vitamins and Clinical Nutrition, Hoffmann-La Roche, Nutley (New Jersey 07110, USA)
Key words. Asparaginase; glutaminase; blastogenesis; mitogenesis.

In 1953 J. Kidd reported that guinea pig serum possessed antilymphoma activity[40]. In 1961 J. Broome showed that the enzyme asparaginase, a normal component of guinea pig serum, was the active antilymphoma agent[17]. This discovery quickly led to the introduction of asparaginase as a remission-inducing agent for human acute lymphocytic leukemia.

Clinical use of asparaginase revealed a variety of side-effects, among which was immunosuppression[46,47,49]. In 1969 G. Astaldi and co-workers showed that asparaginase was a potent inhibitor of mitogen-stimulated lymphocyte blastogenesis[8]. The inhibition of lymphocyte activity is by no means a trivial or unimportant phenomenon and Astaldi's initial report stimulated a considerable amount of research. Between February 1969 when Astaldi's first report appeared and 1974 some 36 papers exploring the phenomenon were published. After 1974 it appears that work in this area came to a virtual end. A single study was published in 1976; we know of no additional publications in subsequent years (table 1).

It is unusual to be able to define precisely the entire literature on a subject in the biological sciences. In the present review we will describe what is known about asparaginase's ability to inhibit the in vitro activity of lymphocytes. We will discuss the 3 hypotheses that have been developed to explain the asparaginase-mediated inhibition of mitogenesis. We will argue that none of these hypotheses is correct. We will also describe our own recent work and briefly present a new hypothesis that attempts to explain why asparaginase is capable of inhibiting lymphocyte activity in vitro.

We begin with a description of the phenomenon. Lymphocytes from humans, mice, rats, rabbits, dogs, and guinea-pigs are inhibited in vitro by asparaginases. Peripheral, splenic, and lymph node lymphocytes appear to be equally sensitive. We know of no reports dealing with thymic lymphocytes. Inhibition is obtained regardless of the stimulus for mitogenesis. The responses to Concanavalin A (Con A), Phytohemagglutinin (PHA), pokeweed mitogen (PWM), purified protein derivative (PPD), varidase, sodium periodate, and the mixed (allogeneic) lymphocyte reaction (MLR) are abolished by asparaginases. All of these stimuli activate primarily T lymphocytes. We know of only 2 reports dealing with the effects of asparaginase on the response to lipopolysaccharide (LPS), a mitogen that is presumed to activate only B lymphocytes. One study showed that the responses to LPS are as sensitive to inhibition as the responses to PHA[16]; the other report indicated that LPS responses were less sensitive to asparaginase inhibition[32]. At least 7 different asparaginases with a variety of physical and catalytic properties have been shown to be capable of inhibiting mitogenesis (table 1). One international unit of asparaginase per milliliter of culture medium is capable of almost completely abolishing the mitogenic response. Lower concentrations of activity partially inhibit mitogenesis but there does not appear to be a strictly linear relationship between asparaginase activity in the medium and the extent of mitogenesis when there is less than 1 IU/ml.

A striking characteristic of the phenomenon is that lymphocyte viability is not affected – even after prolonged incubation with a very high concentration of enzyme activity[19,26,48,63]. A number of studies showed that normal, or near normal, mitogenesis can be obtained by simply washing the cells free of asparaginase and reculturing them with fresh mitogen[19,48]. For maximal effectiveness, the asparaginase must be added to the lymphocyte culture within 1–2 h of mitogenic stimulation. Delayed addition of asparaginase results in a decrease in inhibition that parallels the extent of the delay[19,48]. After about 24 h the addition of asparaginase does not appear to affect the mitogenic response. The addition to the culture of asparagine results in a partial relief of inhibition; we know of no study that has shown that the inhibitory action of asparaginase could be completely reversed by the addition of any compound to the culture. Table 1 summarizes the literature on the ability of asparaginase to inhibit mitogenesis. With the above discussion as background we will present and analyze the 3 hypotheses that have been developed to explain the inhibition of motogenesis by asparaginase. We will discuss these hypotheses in the light of other research that was published simultaneously or subsequently and we will show that none of the 3 hypotheses is likely to be correct.

Asparagine depletion

The most obvious explanation for the ability of asparaginase to inhibit mitogenesis is the hydrolysis of the asparagine present in the culture medium. This hypothesis assumes that asparagine, a nutritionally nonessential amino acid, is essential for mitogenesis. A review of the literature reveals that the amino acid requirements of activated lymphocytes were being explored at the same time that the effects of asparaginase were being investigated. The 2 literatures developed independently; their only point of intersection was an erroneous report[62], subsequently corrected by its authors[61], that asparagine was essential for blastogenesis. The incorrect report specifically dealt with asparaginase and blastogenesis and was so titled. The correction appeared in a paper dealing with the amino acid requirements of lym-

The literature on the effects of asparaginase on mitogenesis

Authors	Cell type	Stimulus	Asparaginase	Explanation
1969				
Astaldi et al.[8]	NHPBL	PHA	*E. coli*	Asn depletion
McElwain and Hayward[43]	LHPBL	PHA	*E. coli*	
Dartnall and Baike[22]	NHPBL	PHA	None	
Astaldi et al.[7]	NHPBL	PHA	*E. coli*	Asn, Gln depletion
Weiner et al.[62]	NHPBL	PHA	None	Asn deficient
Astaldi et al.[9]	NHPBL	MLR	*E. coli*	
Dartnall[21]	NHPBL(?)	PHA	*E. coli*(?)	Asn depletion and other
Simberkoff et al.[56]	NHPBL	PHA	*E. coli*, Agouti	
Calabri et al.[20]	N + LHPBL	PHA	*E. coli*	
1970				
Kirchner and Bauer[41]	NHPBL	PHA	*E. coli*	
Simberkoff and Thomas[57]	NHPBL	PHA	*E. coli*	Gln depletion
Burgio et al.[19]	NHPBL	PHA, PWM, MLR	*E. coli*	
Ohno and Hirsch[48]	C + LHPBL	PHA, Strepto Varidase Vaccina	*E. coli*	Asn, Gln depletion
Ohno and Hirsch[47]	C + NHPBL	PHA	*E. coli*	
Maral et al.[44]	Dog PBL	PHA, MLR	*E. coli*	
Miura et al.[45]	NHPBL	PHA	*E. coli*, guinea pig	Asn or Gln depletion
1971				
Shons et al.[55]	NHPBL	MLR	*E. coli*	
Fidler et al.[29]	Rat PBL	PHA	*E. coli*	
Jasin and Prager[38]	Rat spleen	PHA	*E. coli*	
Giraudo-Conesa et al.[31]	N + LHPBL	PHA	*E. coli*	
Weksler and Weksler[63]	NHPBL	PHA	*E. coli*	Asn depletion
Burgio et al.[18]	N + LHPBL	MLR	*E. coli*	
Astaldi[5]				Review article
Alexander et al.[3]	NHPBL	PHA, Con A, MLR	*E. coli*, Agouti	Asn depletion
Fink et al.[30]	NHPBL	PHA	*E. coli*	
Astaldi et al.[10]				Review article
1972				
Soru et al.[58]	Rabbit spleen	PHA	*Mycobacterium bovis*	Asn depletion
Astaldi[6]				Review article
Schrek et al.[52]	NHPBL tonsil, lymph node	PHA	Bacterial glutaminase/ asparaginase	Gln depletion
Han and Ohnuma[33]	NHPBL	PHA, Con A, PWM, PPD, MLR, staphlococcal filtrate, varidase	*E. coli* *Erwinia carotovora*	
Benezra et al.[15]	NHPBL mouse spleen	PHA	*E. coli*	Gln depletion
Fidler and Montgomery[26]	Rat spleen	Con A	*E. coli*	Membrane effects
1973				
Kahn and Fidler[39]	Rat PBL	MLR	*E. coli*	Membrane effects
Fidler et al.[28]	Rat spleen and lymph node	Con A	*E. coli*	Membrane effects
Berenbaum et al.[16]	Mouse PBL	PHA, LPS	*E. coli*	
1974				
Ashworth and MacLennon[4]	Rabbit PBL	PHA	*E. coli* *Erwinia carotovora*	Gln depletion
1976				
Gordon et al.[32]	Mouse spleen	PHA, Con A, LPS, $NaIO_4$	*E. coli*	Asn depletion

Abbreviations used: Cell types: NHPBL, normal human peripheral blood lymphocytes; LHPBL, leukemic human peripheral blood lymphocytes; CHPBL, human peripheral blood lymphocytes from non-leukemic cancer patients.
Stimuli: PHA, phytohemagglutinin; MLR, mixed (allogenic) lymphocyte reaction; PWM, poke weed mitogen; Strepto, streptokinase-streptodornase; Con A, Concanavalin A; PPD, purified protein derivative; LPS, lipopolysaccharide.
Explanation: Asn, asparagine; Gln, glutamine.

phocytes: neither asparaginase nor blastogenesis appeared in the title. As recently as 1982 the incorrect report was cited to support the hypothesis that asparagine depletion was responsible for the inhibition of mitogenesis[50].

A review of the literature makes it clear that asparagine is not required for mitogenesis[24,25,45,53,61], and therefore the asparagine depletion hypothesis cannot be correct. It is interesting to note that within a few months of Astaldi et al.'s first report, Dartnall and Baikie[22] presented data showing that neither asparagine nor aspartate is required for mitogenesis. It appears that this early report was correct.

Measurements of asparagine synthetase levels of lymphoid tissues have been used to support the asparagine depletion hypothesis. Prager and Derr[51] reported extremely low levels of synthetase activity. However, an earlier report from Meister's laboratory[36] reported values 100 times higher than those of Prager and Derr, though still low when compared to other tissues. That mitoge-

nesis occurs normally in asparagine-free media suggests that the asparagine synthetase levels of lymphocytes are adequate. We cannot, however, rule out the possibility that sub-populations within the total lymphocyte population pool differ in their synthetase levels and that cross-feeding may occur. We also cannot rule out selective protein turnover within lymphocytes as a source of the asparagine required for mitogenesis, though we believe this to be unlikely.

Glutamine depletion

Most asparaginases possess some degree of glutaminase activity. Consequently glutamine depletion was an hypothesis put forward to explain asparaginases' ability to inhibit mitogenesis.

To disprove this hypothesis it is only necessary to show that mitogenesis can be inhibited by an asparaginase that lacks glutaminase activity. This has been demonstrated for the glutaminase-free asparaginases from guinea pig serum[45], agouti serum[3,56], and *Mycobacterium bovis*[58].

Though the glutamine depletion hypothesis is untenable, a review of the studies that supported it is still worthwhile. We now know that lymphocytes require glutamine[24,25,45,53,59,61]. Tissue culture media are routinely supplemented with glutamine, usually to the level of 2 μmoles/ml. A review of the studies that supported the glutamine depletion hypothesis reveals that *E. coli* asparaginase was used as a reagent to selectively deplete the serum component of the media of asparagine and glutamine[15,57].

The depleted media were shown to be incapable of supporting blastogenesis. Supplementation of the depleted medium with asparagine did not restore its ability to support lymphocyte activation; supplementation with glutamine, however, fully restored the medium's effectiveness. Though these studies were performed with the intention of determining why asparaginase inhibits mitogenesis we now recognize that they in fact helped to establish that asparagine is not required for mitogenesis, but glutamine is required. The use of asparaginase to deplete a medium prior to the addition of lymphocytes is not the same as including asparaginase in the medium during the culture period. Simberkoff and Thomas recognized this[57]. They specifically pointed out that glutamine supplementation was effective after heat inactivation of the asparaginase but that no supplementation was effective if active enzyme was present during cultivation. Given the fact that glutamine depletion is not the cause of asparaginases' inhibitory action, we can ask the related question of whether the glutaminase activity can at least contribute to the inhibitory potency of asparaginases. The answer appears to be yes, though the mechanism may be more than depletion of a required nutrient. Two studies comparing the inhibitory effectiveness of the asparaginases form *E. coli* (2–4% glutaminase activity) and *Erwinia carotovora* (8–10% glutaminase) found that the *Erwinia* enzyme was the more potent inhibitor of mitogenesis by a factor of 10. Ashworth and MacLennon[4] attributed the increased potency to the glutaminase activity. Han and Ohnuma[33] however rejected this explanation when they found that supplementation with glutamine did not alleviate the inhibition, whereas supplementation with asparagine partially alleviated the inhibition. Han and Ohnuma attributed the increased potency of the *Erwinia* enzyme to some (unspecified) molecular characteristic of the enzyme molecule. In another study, Schrek et al.[52] determined the inhibitory potency of a bacterial enzyme that hydrolyzes both glutamine and asparagine at similar rates[35]. They found that the dual specificity enzyme was a much more potent inhibitor of mitogenesis than is the *E. coli* enzyme. The difference was assumed to be due to rapid glutamine depletion by the dual specificity enzyme.

To evaluate these studies it is necessary to discuss the mechanisms by which glutamine can be decomposed. The well-known instability of glutamine is not the result of the molecule's tendency to hydrolyze, but rather is due to an exchange reaction between the alpha-amino group and the terminal amide nitrogen. The effect is to displace to the amide nitrogen as a free ammonium ion and convert the rest of the molecule to the cyclic compound 2-pyrrolidone-5-carboxylic acid[60]. This compound has been shown to be nontoxic to lymphocytes in culture[11]. The breakdown of glutamine catalyzed by asparaginases is via hydrolysis of the amide group with the production of an ammonium ion and a molecule of glutamate. Glutamate, we now recognize, is a physiologically active compound that serves many roles besides that of a component of proteins and a biosynthetic intermediate[64]. It is, therefore, possible that the increased inhibitory potency of the *Erwinia* and dual specificity enzymes is due to a combination of glutamine depletion and toxicity of accumulated glutamate. In a series of preliminary experiments we have found that glutamate does in fact inhibit mitogenesis when added to serum supplemented Minimal Essential Medium (unpublished data). It thus seems likely that the mechanism by which glutamine hydrolyzing asparaginases inhibit mitogenesis is complex and that these enzymes may not be the appropriate tools to study the phenomenon.

Membrane effects

Asparaginase mediated alterations of the lymphocyte membrane have been suggested as being responsible for the inhibition of mitogenesis. Lajolo et al.[42] reported that after they incubated lymphocytes with asparaginase the migration of the cells in an electric field was significantly reduced in comparison to control lymphocytes. This change was attributed to alterations of the molecules in the cell membrane. In a series of studies, Fidler and his colleagues[26–29,39] have presented evidence for decreases in the binding of IgG, alloantibodies, Con A, and antigens to the lymphocyte surface following brief incubation with *E. coli* asparaginase. These changes were attributed to an asparaginase-mediated alteration of the topography of the lymphocyte membrane. Evidence was also presented that asparaginase neither sticks to the lymphocyte membrane nor enters the cells[26].

It is difficult to interpret these studies since none of the membrane changes can be directly attributed to any of

asparaginase's known catalytic activities. Glycoproteins in the cell membrane were assumed to be involved in the inhibitory mechanism. These molecules often serve as receptors, and the oligosaccharide portion is usually attached to the protein portion of the molecule by an N-asparaginyl linkage. There was an early report that asparaginase could cleave preformed glycoproteins; however, Fidler and Montgomery were unable to reproduce these findings[28]. Asparagine-linked glycoproteins possess a predictable sequence of amino acids on either side of the oligosaccharide bearing asparagine residue[54]. However asparaginases do not hydrolyze peptide bonds. Studies of the mechanism of the asparaginase catalytic site have shown that a free alpha carboxyl group is necessary for binding and catalysis[37]. It has also been shown that binding of the amide nitrogen to the asparaginase active site is very sensitive to steric interference: a substituent as small as an N-methyl group abolishes catalytic activity[34]. Given the large size of the oligosaccharide bound to the asparaginyl residue of glycoproteins it is not likely there is any binding of this residue to the asparaginase active site. Jasin and Prager have presented evidence that in vitro glycoprotein synthesis is more sensitive to inhibition by asparaginase than is general protein synthesis[38]. It seems reasonable to expect that cells that have grown in an organ or medium that contained asparagine would exhibit a lag phase of suddenly deprived of asparagine by the addition of asparaginase. But given the fact that normal mitogenesis occurs in asparagine-free media a transient lag is all that should be observed following the addition of asparaginase to culture media.

As part of our own recent reexamination of asparaginase's ability to inhibit mitogenesis we have utilized the asparaginase produced by the bacterium *Vibrio succinogenes*[13,14,23]. We have taken advantage of the recently developed technology of covalent modification of enzymes with methoxypolyethylene glycol (PEG). PEG-asparaginases are nonimmunogenic and nonantigenic[1,2,12]. That is, they do not bind to antibodies specific for the native (non-modified) enzyme and do not induce antibodies against themselves. These effects are due to steric shielding of the enzyme within a sphere of the linear (mol.wt = 5000), hydrophilic PEG molecules. That these modified enzymes are not processed by macrophages, and do not bind to antibodies suggested to us that if the membrane alteration hypothesis were correct. PEG-asparaginases should not inhibit mitogenesis. The effectiveness of the steric shielding provided by the PEG-shell is such that contact of the asparaginase active site with the lymphocyte membrane is extremely unlikely. We have found that rat and guinea pig lymphocytes are inhibited by the PEG-modified *V. succinogenes* asparaginase. We interpret our data as effectively ruling out the membrane alteration hypothesis[13,14]. We cannot explain the evidence for asparaginase-mediated alterations of the lymphocyte membrane. Unless all asparaginases have a yet to be discovered additional catalytic activity, it appears that the effects that have been reported by Fidler and his colleagues[26-29,39] and by Lajolo et al.[42] are not likely to be the primary asparaginase-mediated event.

We believe that the literature we have reviewed is essentially all that is available on the subject. Our analysis of this literature convinces us that the research ended prematurely and that the phenomenon has yet to be explained. It is interesting to note that of the 37 papers summarized in the table only 4 appeared in immunology journals. Perhaps this is the reason that the phenomenon has been overlooked. Our own recent work has led us to propose that asparaginase inhibits mitogenesis by binding a lymphokine or other mitogenic factor. We base this hypothesis on our present knowledge of the binding characteristics of the asparaginase active site and the fact that the temporal pattern of asparaginase sensitivity coincides with the period during which mitogenic factors are secreted by stimulated lymphocytes. Our hypothesis has yet to be tested. We hope that our recent work and the present review will serve to stimulate interest in this overlooked area.

Acknowledgments. This work was supported, in part, by a grant from the Charles and Johanna Busch Foundation (Rutgers University).

* Author for correspondence.

1 Abuchowski, A., McCoy, I.R., Palczuk, N.C., Van Es, T., and Davis, F.F., Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J. biol. Chem. *252* (1977) 3582–3586.

2 Abuchowski, A., Van Es, T., Palczuk, N.C., and Davis, F.F., Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. J. biol. Chem. *252* (1977) 3578–3581.

3 Alexander, P., Delorme, E.J., Currie, A., and Sime, G.C., Inhibition of immunoblast response *in vitro* and *in vivo* by asparaginase from *E. coli* – and Agouti serum in: La-L-asparaginase, pp. 189–193. Eds J. Bernard and M. Boiron. Editions Du Centre National de la Recherche Scientifique, Paris 1971.

4 Ashworth, L.A.E., and MacLennon, A.P., Comparison of the L-asparaginases from *Escherichia coli* and *Erwinia carotovora* as immunosuppressants. Cancer Res. *34* (1974) 1353–1359.

5 Astaldi, G., On the so-called antiblastic activity of L-asparaginase. Boll. Ist. Sieroter. Milan *50* (1971) 278–290.

6 Astaldi, G., Is L-asparaginase an antiblastic agent? Haematologia *6* (1972) 433–446.

7 Astaldi, G., Burgio, G.R., Biscatti, G., Astaldi, A., and Ferfoglia, L., Immunosuppressive action of L-asparaginase. Lancet *2* (1969) 643.

8 Astaldi, G., Burgio, G.R., Krc, J., Genova, R., and Astaldi, A.A., L-asparaginase and blastogenesis. Lancet *1* (1969) 423.

9 Astaldi, G., Micu, D., Astaldi, A., and Burgio, G.R., Immunosuppressive action of L-asparaginase. Lancet *2* (1969) 1357.

10 Astaldi, G., Micu, D., Astaldi, A., Burgio, G.R., and Krc, I., Further investigation on L-asparaginase and immune reactions, in: La-L-asparaginase, pp. 205–219. Eds J. Bernard and M. Boiron. Editions Du Centre National de la Recherche Scientifique, Paris 1971.

11 Baechtel, S., Greg, D.E., and Prager, M.D., The influence of glutamine, its decomposition products and glutaminase on the transformation of human and mouse lymphocytes. Biochim. biophys. Acta *421* (1976) 33–43.

12 Bendich, A., Kafkewitz, D., Abuchowski, A., and Davis, F.F., Immunological effects of native and polyethylene glycol modified asparaginases from *Vibrio succinogenes* and *Escherichia coli* in normal and tumor-bearing mice. Clin. exp. Immun. *48* (1982) 273–278.

13 Bendich, A., Kafkewitz, D., Abuchowski, A., and Davis, F.F., Inhibition of blastogenesis by native and polyethylene glycol-modified asparaginases from *Escherichia coli* and *Vibrio succinogenes*. Immun. Comm. *12* (1983) 273–284.

14 Bendich, A., Kafkewitz, D., Abuchowski, A., and Davis, F.F., Inhibition of guinea pig lymphocyte blastogenesis by polyethylene glycol-modified asparaginases and a new hypothesis to explain this phenomenon. (1983) submitted.

15 Benezra, D., Pitaro, R., Birkenfeld, V., and Hochman, A., Reversal of the immuno-suppressive effect of L-asparaginase by L-glutamine. Nature, New Biol. *236* (1972) 80–82.

16 Berenbaum, M.C., Cope, W.A., and Jeffery, N., Differential asparaginase sensitivity of T-cell and B-cell responses. Clin. exp. Immun. *15* (1973) 565–572.
17 Broome, J.D., Evidence that the L-asparaginase activity of guinea pig serum is responsible for its antilymphoma effects. Nature *191* (1961) 1114–1115.
18 Burgio, G.R., Astaldi, A., Vaccaro, R., and Gasporini, M.C., Über die immunsuppressiven Eigenschaften der L-asparaginase. Mschr. Kinderheilk. *119* (1971) 439–442.
19 Burgio, G.R., Astaldi, A., Krc, I., Micu, D., and Astaldi, G., With references to blastogenesis inhibition by L-asparaginase, in: Recent Results in Cancer Research, pp. 288–295. Eds E. Grundmann and H.F. Oettgen. Springer-Verlag, New York 1970.
20 Calabri, G., Gatta, A., and Guazzelli, C., Inibizione della blastogenesi infocitaria *in vitro* con L-asparaginasi in soggetti normali e leucemici. Haematologica *54* (1969) 890–898.
21 Dartnall, J.A., L-asparaginase and blastogenesis. Lancet *2* (1969) 1357–1358.
22 Dartnall, J.A., and Baikie, A.G., L-asparaginase and blastogenesis. Lancet *1* (1969) 1098.
23 Distasio, J.A., Niederman, R.A., Kafkewitz, D., and Goodman, D., Purification and characterization of L-asparaginase with antilymphoma activity from *Vibrio succinogenes*. J. biol. Chem. *251* (1976) 6929–6933.
24 Doherty, N.S., and Machin, P., Investigations into the *in vitro* and *in vivo* immunosuppressant activity of N-acetyl-3-sulphonamoly-L-alanine methyl ester, a potential L-asparagine antimetabolite. Biochem. Pharm. *29* (1980) 1767–1771.
25 Dubrow, R., Pizer, L.I., and Brody, J.I., Serine-glycine requirement of phytohemagglutinin stimulated lymphocytes. J. natl Cancer Inst. *51* (1973) 307–311.
26 Fidler, I.J., and Montgomery, P.C., Effects of L-asparaginase on lymphocyte surface and blastogenesis. Cancer Res. *32* (1972) 2400–2406.
27 Fidler, I.J., and Montgomery, P.C., Effects of L-asparaginase on lymphocyte's surface leading to decreased binding of alloantibodies. Res. Commun. Chem. Path. Pharm. *5* (1973) 627–634.
28 Fidler, I.J., Montgomery, P.C., and Cesarini, J.P., Modification of surface topography of lymphocytes by L-asparaginase. Cancer Res. *33* (1973) 3176–3180.
29 Fidler, I., Montgomery, P.C., and Ferguson, E., L-asparaginase and lymphocyte blastogenesis. Lancet *2* (1971) 1091–1092.
30 Fink, U., Brintigger, G., Konig, E., Cohnen, G., Hanke, U., and Begemann, H., Inhibition by L-asparaginase of incorporation of labelled DNA, RNA and protein precursors into human peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA) *in vitro*, in: La-L-asparaginase, pp. 199–203. Eds J. Bernard and M. Boiron. Editions Du Centre National de la Recherche Scientifique, Paris 1971.
31 Giraudo-Conesa, L.C., Parlovsky, S., and Scornik, J.C., Inhibition of lymphocyte transformation by L-asparaginase in acute leukemia. Medicine (Buenos Aires) *31* (1971) 434–439.
32 Gordon, W.C., Mandy, W.J., and Prager, M.D., Differential effect of asparaginase on lymphocyte subpopulations and alleviation of the immunosuppression by lipopolysaccharide. Cancer Immun. Immunother. *1* (1976) 205–209.
33 Han, T., and Ohnuma, T., *In vitro* blastogenesis inhibited by *Erwinia carotovora* L-asparaginase. Nature, New Biol. *239* (1972) 50–51.
34 Herrmann, V., Rohm, K.H., and Schneider, F., On the substrate specificity of L-asparaginase from *E. coli*. FEBS Lett. *39* (1974) 214–217.
35 Holcenberg, J.S., Therapy of neoplasia with other nonessential amino acid degrading enzymes, in: Enzymes as Drugs, p. 38. Eds J.S. Holcenberg and J. Roberts. John Wiley and Sons, New York 1981.
36 Horowitz, B., Madras, B.K., Meister, A., Old, L.J., Boyse, E.A., and Stokert, E., Asparagine synthetase activity of mouse leukemias. Science *160* (1968) 533–535.
37 Howard, J.B., and Carpenter, F.H., L-asparaginase from *Erwinia carotovora*. J. biol. Chem. *247* (1972) 1020–1030.
38 Jasin, H.E., and Prager, M.D., Effects of L-asparaginase on *in vitro* immunoglobulin synthesis by rat spleen cells. Clin. exp. Immun. *9* (1971) 515–523.
39 Kahn, J.M., and Fidler, I.J., L-asparaginase and alteration of lymphocyte surface. J. Med. *4* (1973) 248–256.
40 Kidd, J.G., Regression of transplanted lymphomas induced *in vivo* by means of normal guinea pig serum I. Course of transplanted cancers of various kinds in mice and rats given guinea pig serum, horse serum or rabbit serum. J. exp. Med. *98* (1953) 568–582.
41 Kirchner, H., and Bauer, S., Wirkung von L-asparaginase aus *Escherichia coli* auf phytohamagglutinin-stimulierten Lymphocyten Kulturen. Klin. Wsch. *48* (1970) 50–51.
42 Lajolo, D., Astaldi, A., Pecco, P., Bert, G., and Astaldi, G., Electrophoretic mobility of human lymphocytes in the presence of *E. coli* L-asparaginase. Exp. Cell Res. *60* (1970) 458–459.
43 McElwain, T.J., and Haward, S.K., L-asparaginase and blastogenesis. Lancet *1* (1969) 527.
44 Maral, R., Guyonnet, J.C., Julou, L., de Ratuld, Y., and Werner, G.H., Studies on the immunosuppressive activity of L-asparaginase, in: Recent Results in Cancer Research, pp. 160–173. Eds E. Grundmann and H.F. Oettgen. Springer-Verlag, New York 1970.
45 Miura, M., Hirano, M., Kakizawa, K., Morita, A., Uetani, T., and Yamada, K., Inhibitory effect of L-asparaginase in lymphocyte transformation induced by phytohemagglutinin. Cancer Res. *30* (1970) 768–772.
46 Oettgen, H.F., L-asparaginase: Current studies of clinical evaluation, in: Antineoplastic and Chemotherapeutic Agents, Part II, p. 423–742. Eds A.C. Santotelli and D.G. Johns. Springer-Verlag, New York 1975.
47 Ohno, R., and Hersh, E.M., Immunosuppressive effects of L-asparaginase. Cancer Res. *30* (1970) 1605–1611.
48 Ohno, R., and Hersh, E.M., The inhibition of lymphocyte blastogenesis by L-asparginase. Blood *35* (1970) 250–262.
49 Pappas, A., Hirschmann, W.D., Scheurlen, P.G., and Esser, W., Hemmung der Lymphozytentransformation in der Diffusionskammer ('in vivo') durch L-asparaginase. Medizinische Welt *2* (1970) 1288–1291.
50 Prager, M.D., Baechtel, F.S., and Heifetz, A., Antitumor and other effects of asparaginase, in: Bacteria and Cancer, pp. 291–306. Eds J. Jepjaszewicz, G. Pulverer and W. Roszkowski. Academic Press, New York 1982.
51 Prager, M.D., and Derr, I., Metabolism of asparagine, aspartate, glutamine, and glutamate in lymphoid tissue: basis for immunosuppression by L-asparaginase. J. Immun. *106* (1971) 975–979.
52 Schrek, R., Batra, K.V., Holcenberg, J.S., Roberts, J., and Dolowy, W.C., Effect of L-glutaminase on transformation and DNA synthesis of normal lymphocytes. Acta haemat. *48* (1972) 12–15.
53 Schrek, R., Holcenberg, J.S., Batra, K.V., Roberts, J., and Dolowy, W.C., Effect of asparagine and glutamine deficiency on normal and leukemic cells. J. natl Cancer Inst. *51* (1973) 1103–1107.
54 Sharon, N., Complex Carbohydrates, p. 109. Addison Wesley Publ. Co., Reading, Mass. 1975.
55 Shons, A.R., Etheredge, E.E., and Najarian, J.S., The effect of L-asparaginase in mixed lymphocyte culture. Transplantation *12* (1971) 85–87.
56 Simberkoff, M.S., Thorbecke, G.J., and Thomas, L., Studies of PPLO infection V. Inhibition of lymphocyte mitosis and antibody formation by mycoplasmal extracts. J. exp. Med. *129* (1969) 1163–1181.
57 Simberkoff, M.S., and Thomas, L., Reversal by L-glutamine of the inhibition of lymphocyte mitosis caused by *E. coli* asparaginase. Proc. Soc. exp. Biol. Med. *133* (1970) 642–644.
58 Soru, E., Teodorescu, M., Zaharia, B., Szabados, J., and Rudescu, K., L-asparaginase from the BCG strain of *Mycobaterium bovis*. Can. J. Biochem. *50* (1972) 1149–1157.
59 Taudou, D.G., Wiart, J., Taudou, B., and Panijel, J., Influence of certain amino acids in steps leading to DNA synthesis in concanavalin A-treated guinea-pig lymphocytes. Ann. Immun. (Inst. Pasteur) *128* (1977) 1065–1077.
60 Waelsch, H., Certain aspects of intermediary metabolism of glutamine, asparagine and glutatione, in: Advances in Enzymology, pp. 237–319. Ed. F.F. Ford. Interscience Publ. Ltd, London 1952.
61 Waithe, W.I., Dauphinais, C., Hathaway, P., and Hirschorn, K., Protein synthesis in stimulated lymphocytes II amino acid requirements. Cell. Immun. *17* (1975) 323–334.
62 Weiner, M.S., Waithe, W.I., and Hirschorn, K., L-asparaginase and blastogenesis. Lancet *2* (1969) 748.
63 Weksler, M.E., and Weksler, B.B., Studies on the immunosuppressive properties of asparaginase. Immunology *21* (1971) 137–150.
64 White, A., Handler, P., Smith, E.L., Hill, R.L., and Lehman, I.R., Principles of Biochemistry, 6th edn, p. 1115. McGraw Hill, New York 1978.

0014-4754/84/111173-05$1.50 + 0.20/0
© Birkhäuser Verlag Basel, 1984