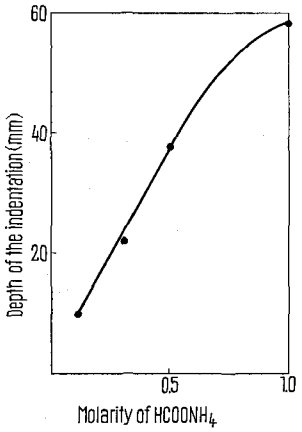

Fig. 2. Dependence of the depth of the indentation of poly C on the concentration of ammonium formate. $1.7 \times 10^{-5} M$ poly C in $0.1 M$ sodium phosphate with ammonium formate in the concentration given in the graph (pH 7).

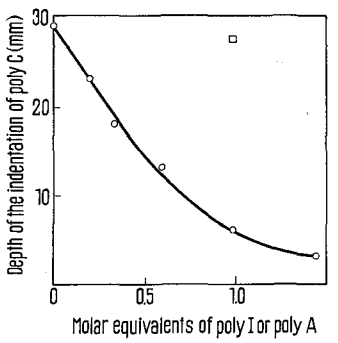

Fig. 3. Formation of the 1:1 complex of poly (C) × poly (I) followed by oscillopolarographic technique. Homopolymers were mixed in $0.1 M$ NaCl with $0.01 M$ sodium phosphate (pH 7). After 2 h of incubation at room temperature, the supporting background electrolyte was added. The oscillopolarographic measurements were carried out in $0.3 M$ ammonium formate with $0.1 M$ sodium phosphate pH 7. The depth of the indentation of poly C was measured on the first curve. Concentration of poly C ($7.5 \times 10^{-5} M$) was held constant in all samples, while the amount of poly I (○——○) or poly A (□) varied as indicated in the Figure.

nected with single-strand breaks and/or other irregularities present in the double-helical structure of poly (I) · poly (C), as is the case with DNA[9]. The mixing of poly C with polyadenylic acid (poly A) had almost no influence on the depth of the indentation of poly C (Figure 3) (poly A does not form a complex with poly C under the given conditions[6]).

Our preliminary results[10] show that polarographic techniques may become useful in the study of the structure of synthetic polynucleotides. A paper concerning the character of processes to which poly C and other polynucleotides are subject on the electrode will be published elsewhere.

Zusammenfassung. Es ergibt sich, dass die Polyzytidylsäure unter dem neutralen pH eine polarographische Reduktionswelle ähnlich der Welle der denaturierten Desoxyribonukleinsäure gewährt. Zur Bestimmung der Polyzytidylsäure mit der «ersten Kurve» genügt bereits 1/10 μg des Stoffes. Im Komplex der Polyzytidylsäure mit Polyinosinsäure ist die Reduzierbarkeit der Polyzytidylsäure eliminiert.

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[9] E. PALEČEK, Biochim. biophys. Acta *145*, 410 (1967).
[10] E. PALEČEK, unpublished.

Anti-Tumour Activity of Carbobenzoxy-L-Asparagine

Various neoplasms in different animal species are inhibited by treatment with L-asparaginase derived from either guinea-pig serum or *Escherichia coli*[1-5]. Certain human leukemias were also found to be sensitive to treatment with the bacterial enzyme[6]. This effect was ascribed to the enzymatic deamidation of L-asparagine, an amino acid essential for the growth of the susceptible tumours[7]. Since it is possible to prevent utilization of nutrilites by structural analogues, it was felt that compounds structurally related to L-asparagine might also inhibit the growth of L-asparaginase-sensitive tumours. Various L-asparagine analogues were found to inhibit L-asparaginase activity of *Mycobacterium phlei*[8] and of rat liver[9]. The L-asparaginase activity of *Saccharomyces cerevisiae* was previously found to be competitively inhibited by carbobenzoxy-L-asparagine[10]. The present communication describes the effect of the latter compound on the growth of an L-asparaginase-sensitive murine lymphoma.

Carbobenzoxy-L-asparagine (CBZ-asparagine) was purchased from Fluka AG, Buchs, Switzerland. A fine suspension was obtained by homogenizing it in normal saline with mortar and pestle. A single injection of CBZ-asparagine at doses up to 120 mg per mouse did not show any noticeable toxic effects. For treatment of

[1] J. G. KIDD, J. exp. Med. *98*, 569 (1953).
[2] J. D. BROOME, J. exp. Med. *118*, 99 (1963).
[3] E. A. BOYSE, L. J. OLD, H. A. CAMPBELL and L. T. MASHBURN, J. exp. Med. *125*, 17 (1967).
[4] L. T. MASHBURN and J. C. WRISTON, Archs Biochem. Biophys. *105*, 450 (1964).
[5] L. J. OLD, E. A. BOYSE, H. A. CAMPBELL, R. S. BRODLEY, J. FIDLER and J. D. TELLER, Cancer *20*, 1066 (1967).
[6] J. M. HILL, J. ROBERTS, E. LOEB, A. KAHN, A. MACLELLAN and R. W. HILL, J. Am. Med. Ass. *202*, 116 (1967).
[7] J. D. BROOME, J. exp. Med. *118*, 121 (1963).
[8] Y. S. HALPERN and N. GROSSOWICZ, Biochem. J. *65*, 716 (1957).
[9] N. DE GROOT and N. LICHTENSTEIN, Biochim. biophys. Acta *40*, 92 (1960).
[10] O. YARON-GIZRI, M. Sc. Thesis, The Hebrew University, Jerusalem (1963).

tumour-bearing mice a total of 15 i.p. injections were administered, each consisting of 10 mg CBZ-asparagine in 0.1 ml. Injections were given 6 days a week, starting 1 day after tumour implantation.

The tumour used was a lymphosarcoma induced in SJL/J mice by treatment with 7,12-dimethyl-benz-[α]-anthracene[11]. The tumour was kept by serial transplantations in inbred mice of the SJL/J strain, bred at the Department of Experimental Medicine and Cancer Research. The lymphoma formed large s.c. tumours upon s.c. inoculation. Treatment of the host with guinea-pig serum was found to inhibit tumour growth completely.

The effect of CBZ-asparagine was tested in SJL/J mice which received a s.c. inoculum of 1×10^6 tumour cells suspended in 0.2 ml normal saline.

Progressively growing tumours developed in all animals, both untreated and treated. However, the growth rate of the tumour was different in the 2 groups. In untreated mice s.c. tumours became palpable after an average of 9.1 days in females, and 11.6 days in males (Table I). In CBZ-asparagine-treated mice tumours became palpable markedly later (Table I). The difference between the time of appearance of the tumour in treated and untreated females was statistically significant ($p < 0.02$) as was the difference between males of the 2 groups ($p < 0.02$). The survival time of treated males was slightly prolonged in comparison with untreated mice ($0.05 > p > 0.02$). No significant prolongation of the survival of treated females was noted.

In a subsequent experiment male mice were killed 18 days after tumour inoculation, the tumours were carefully excised and weighed (Table II). The tumour weights in treated animals were markedly lower than those in untreated controls ($p < 0.01$).

The results of the present study show that CBZ-asparagine, at the treatment-schedule employed, has a marked tumour-inhibitory effect. It remains to be seen whether different treatment schedules would show a stronger inhibition than that obtained; the effect on other transplantable tumours will also be studied.

S-carbamyl-L-cysteine, which can be looked at as an L-asparagine analogue, was found to be effective against several tumours; this compound was equally effective on L-asparaginase-sensitive and resistant tumours[12]. It should be noted however that S-carbamyl-L-cysteine may also be regarded as a glutamine analogue[13]. Recently 5-diazo-4-oxo-L-norvaline (DONV), another L-asparagine analogue, was found to inhibit the growth of L-asparagine-dependent tumour cells in culture[14].

As to the mode of inhibition of tumour growth by CBZ-asparagine the following possibilities should be taken into consideration: (a) CBZ-asparagine may interfere with the utilization of L-asparagine by tumour cells. (b) CBZ-asparagine interfers with the utilization of glutamine, as this compound inhibits markedly the activity of rat liver NAD-synthetase, rat liver glutaminase, and ovine brain γ-glutamyl transferase[15]. (c) CBZ-asparagine may interfere with both asparagine and glutamine metabolism.

Recently KIM et al.[16] showed that certain HeLa cell lines were sensitive to *E. coli* asparaginase, while they were resistant to the action of guinea-pig serum. Addition of glutamine reversed the effect of the microbial asparaginase, which also has some glutaminase activity, indicating that this enzyme may also affect glutamine utilization[17].

Table I. Effect of CBZ-L-asparagine on the growth of a transplantable lymphosarcoma in SJL/J mice

Sex of host	Treatment	Mean time of tumour appearance (days) ± S.E.[a]	Mean survival time (days) ± S.E.
Male	None	11.6 ± 0.87 (12)[b]	28.8 ± 1.43 (11)[b]
Female	None	9.1 ± 0.65 (11)	21.4 ± 1.05 (9)
Male	CBZ-L-asparagine	16.8 ± 1.59 (11)	34.2 ± 1.75 (9)
Female	CBZ-L-asparagine	11.8 ± 0.66 (10)	24.6 ± 1.10 (8)

[a] The time of tumour appearance was determined by daily manual palpation. [b] No. of mice used.

Table II. The effect of CBZ-L-asparagine on tumour weight

	Tumour weight (g)[a]	
	Untreated control	Treated
Individual tumours	4.86	3.08
	4.38	2.41
	3.53	2.30
	3.42	0.58
	2.92	0.34
	2.69	0.22
	2.00	0.12
	1.05	0.08
	0.90	0.04
Average ± S.E.	2.86 ± 0.46	1.02 ± 0.41

[a] Tumour weight was determined 18 days after s.c. implantation of a transplantable lymphosarcoma to male SJL/J mice.

Résumé. L'administration i.p. de la carbobenzoxy-L-asparagine a retardé la croissance des greffes s.c. d'un lymphosarcome de souris, sensitif à l'action de l'L-asparaginase. L'apparition des tumeurs a été retardée, la période de survie a été prolongée et le poids des tumeurs réduit d'une façon significative chez les animaux traités. Le mécanisme de l'inhibition des tumeurs est considéré.

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6 September 1968.

[11] N. HARAN-GHERA, M. KOTLER and A. MESHORER, J. natn. Cancer Inst. *39*, 63 (1967).
[12] R. H. ADAMSON, Nature *217*, 751 (1968).
[13] J. M. RAVEL, T. J. MCCORD, C. G. SKINNER and W. SHIVE, J. biol. Chem. *238*, 159 (1958).
[14] R. E. HANDSCHUMACHER, C. J. BATES, P. K. CHANG, A. T. ANDREWS and G. A. FISCHER, Science *161*, 62 (1968).
[15] G. MOR, H. SPIEGELSTEIN-KLARFELD and N. LICHTENSTEIN, to be published.
[16] J. H. KIM, E. A. BOYSE, L. J. OLD and H. A. CAMPBELL, Biochim. biophys. Acta *158*, 476 (1968).
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