MATSUI, H. & SCHWARTZ, A. (1966). Purification and properties of a highly active ouabain-sensitive Na+, K++-dependent adenosinetriphosphatase from cardiac tissue. *Biochim. biophys. Acta*, **128**, 380–390.

SANTI, R. (1966). Sulla farmacologia dei flavonoidi. *Atti "Symposium sui Bioflavonoidi"*, ed. Zambotti, V., pp. 58–89. Institute of Biochemistry, University of Milan, Italy.

A possible reaction mechanism for the enzyme histaminase

F. BUFFONI, *Department of Pharmacology, University of Florence, Italy*

Pig plasma histaminase is a copper-pyridoxal-phosphate enzyme (Buffoni & Blaschko, 1964; Blaschko & Buffoni, 1965; Buffoni, 1966) which catalyses the general reaction:

$$R\text{CH}_2\text{NH}_2 + \text{O}_2 + \text{H}_2\text{O} \longrightarrow R\text{COH} + \text{NH}_3 + \text{H}_2\text{O}_2$$

Direct evidence for the formation of a Schiff base between enzyme and substrate in the first step of reaction has been obtained using ^{14}C-histamine. Under anaerobic conditions the enzyme binds 3 moles of histamine per mole of protein, forming an imine bond. The borohydride-reduced Schiff base, pyridoxyl-histamine-5′-phosphate has been isolated and identified by paper and thin-layer chromatography and by fluorescence properties (Buffoni, 1966).

Direct evidence that water is not involved in the ammonia formation has been obtained by using $^3\text{H}_2\text{O}$ and isolating the ammonia formed in the reaction (Buffoni & Della Corte, 1967).

Electron spin resonance (ESR) experiments have shown that the copper remains divalent in the oxidized and reduced enzyme (Buffoni, Della Corte & Knowles, 1968) although there were changes in ESR line shape after reduction and of the ESR signal throughout the catalytical cycle (quench flow method).

These and other experiments on the kinetics of the reaction suggest a mechanism of reaction for the enzyme histaminase in which the Schiff base formation represents the rate limiting step.

REFERENCES

BLASCHKO, H. & BUFFONI, F. (1965). Pyridoxal phosphate as a constituent of the histaminase (benzylamine oxidase) of pig plasma. *Proc. R. Soc. B.*, **163**, 45–60.

BUFFONI, F. (1966). Histaminase and related amine oxidases. *Pharm. Rev.*, **18**, 1163–1200.

BUFFONI, F. (1966). Pyridoxal catalysis in pig plasma benzylamine oxidase (histaminase). *Second Symposium on Chemical and Biological Aspects of Pyridoxal Catalysis. Abstracts*, pp. 20–21.

BUFFONI, F. & BLASCHKO, H. (1964). Benzylamine oxidase and histaminase: purification and crystallization of an enzyme from pig plasma. *Proc. R. Soc.*, **161**, 153–167.

BUFFONI, F. & DELLA CORTE, L. (1967). Sul meccanismo della reazione enzimatica della benzilaminossidasi (istaminasi) del plasma di suino. *Boll. Soc. it. Biol. Sper.*, **43**, 1395–1398.

BUFFONI, F., DELLA CORTE, L. & KNOWLES, P. F. (1968). The nature of copper in pig plasma benzylamine oxidase. *Biochem. J.*, **106**, 557–576.

Immunosuppressive activity of methotrexate and arabinosyl cytosine in mice bearing L1210 leukaemia

L. BALDINI, G. BRAMBILLA, M. CAVANNA* and S. PARODI, *Department of Pharmacology, University of Genoa, Italy*

We have studied the inter-relationship between chemotherapy and immunity in a tumour-host system in which, in addition to the possible tumour-specific antigenicity,

TABLE 1. *Effects of methotrexate (MTX) and arabinosyl cytosine (ara-C) in F_3 male mice inoculated with L1210*

Genotype	Treatments (mg/kg per day × No. injections)	Mortality distribution (days after tumour inoculation) 5–10	11–15	16–20	21–25	26–30	31–40	41–50	Average survival (days)[f]	% Increase avg. survivors over F_3 controls	50-Day survivors (No./total)	50-Day survivors (%)
DBA/2 [a]	Saline	37	3						8·7	—	0/40	0
F_3 [a]	Saline	116	12		1	1			9·4	—	19/149	12·7
F_3 [a]	MTX 0·65 × 4 [e]		56	18	2	1			14·9	58·5	9/86	10·5
F_3 [a]	MTX 0·65 × 12 [d]	1	10	35	6	1	1		17·9	90·4	2/56	3·6
DBA/2 [b]	Saline	7	4						10·2	—	0/11	0
F_3 [b]	Saline	6	21	1					11·6	—	7/35	20·0
F_3 [b]	ara-C 500 × 6 [e]		10	16	11	12	5	2	22·5	94·0	13/69	18·8

(a): Intraperitoneal inoculation of 2×10^6 cells prepared from L1210 infiltrated spleens of DBA/2. (b): Intraperitoneal inoculation of 2×10^3 L1210 ascites cells. (c): Given intraperitoneally once daily starting 3 days after L1210 inoculation. (d): Given intraperitoneally once daily starting 6 days after L1210 inoculation. (e): Given subcutaneously every 4 days starting 6 days after L1210 inoculation. (f): Calculated excluding 50-day survivors.

there is also present the antigenicity of the histocompatibility loci. This was done by transplanting the L1210 leukaemia in mice (F_3) obtained from the mating of the backcrosses (C57BL/6 female × DBA/2 male)F_1 × DBA/2 male. In these animals the proportion of C57BL/6 homozygosis is 1/16 and that of the mice susceptible to the L1210 is therefore $(15/16)^n$ (n = number of histocompatibility loci admitting independent antigenicities).

The results (Table 1) first indicate that treatment with methotrexate (0.65 mg/kg per day for 4 days) or with arabinosyl cytosine, although prolonging the average survival time, does not elicit any increase in the percentage of the 50-day survivors. In mice treated with a higher, but non-toxic, dose of methotrexate (0.65 mg/kg per day for 12 days) the proportion of 50-day survivors was markedly reduced. In both treated and control groups, all the 50-day survivors were resistant to the intraperitoneal reinoculation of 2×10^3 L1210 ascites cells, thus demonstrating that the regression of the leukaemia was brought about by immunological mechanisms.

The results also show that the number of histocompatibility loci which is able to hinder the development of the lymphoma depends on the size of inoculum, being of about two loci in the mice transplanted with 2×10^6 leukaemic spleen cells and of three to four in those inoculated with 2×10^3 L1210 ascites cells.

These results strongly suggest that the immunosuppressive activity of antineoplastic agents can reduce the ultimate therapeutic effects of antitumour treatments.

Structure activity of diuretics as hyperglycaemic agents

A. M. GRANT, S. KREES, J. K. VIKTORA, J. K. WALES, and F. W. WOLFF*, *Research Foundation of the Washington Hospital Center, and Division of Clinical Pharmacology, Department of Medicine, George Washington University School of Medicine, Washington, D.C., U.S.A.*

We have investigated structural changes in the chlorthalidone (1-oxo-3-(3′-sulphamyl-4′-chlorophenyl)-3-hydroxyisoindoline) molecule and have prepared a compound C3/76 (1-oxo-3-(4′-chlorophenyl)-3-hydroxyisoindoline). This is anti-diuretic in the water-loaded rat and shows prolonged and marked hyperglycaemic and insulin inhibiting effects, being in this respect several times more potent than chlorthalidone. Removal of the sulphamyl group led to loss of diuretic activity, and caused anti-diuresis as well as hyperglycaemia associated with insulin inhibition, similar to that found in the development of diazoxide from chlorothiazide.

In our laboratory we have prepared demethylated diazoxide (3-methyl-7-chloro-1,2,4-benzothiadiazine-1,1-dioxide) (AO25), which shows good hyperglycaemic activity and in contrast to diazoxide, little toxicity and no anti-diuretic activity in the rat, both orally and intraperitoneally. It also inhibits insulin secretion *in vitro*. Larger doses of AO25 intravenously in the Rhesus monkey produced hyperglycaemia, reduced plasma insulin level, and a moderate diuresis.

Tolbutamide in the anaesthetized as well as unanaesthetized dog interferes with all three parameters of diazoxide action, on pancreatic islet cells, kidneys, and blood vessels. It also interferes with the action of diazoxide on the isolated rat renal artery. Tolbutamide also interferes with the hyperglycaemic and hypotensive action of AO25. In view of the similarity of tolbutamide and the benzothiadiazines, a competitive inhibition is postulated.