showed the derivatives, which have in the molecule as R_2 a *p*-tolyl group and as R_1 an aromatic ring. For the antiherpetic activity the presence of a halogen atom is not necessary. But if it is, then its place in the molecule is of some importance. 1'-furylidene group as R_1 decreases the antiherpetic activity of the molecule.

It was established for the isatine β-thiosemicarbazone, a compound from the group of related substances-thiosemicarbazones, that the primary site of action lies in the late phases of virus replication[4–6]. About the intracellular site of action of our compounds, we can only suppose from experiments with one of the thiazolidine acetic acid derivatives – DFT, which indicate that the late phases of virus multiplication are affected[7].

Zusammenfassung. Durch Abwandlung der Struktur der 4-Oxo-5-thiazolidinessigsäure ergeben sich starke inhibitorische Effekte auf die cytopathogenen Wirkungen, die vom Virus *Herpes simplex* in Kulturen menschlicher Embryonalzellen ausgeübt werden.

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Histamine Release by Passive Sensitization with Murine Neoplastic Mast Cells in vitro[1]

Histamine release and mast cell damage have been reported recently by Humphrey and Austen[2] and by Keller[3] from the incubation of isolated rat peritoneal mast cells with rabbit antiserum against rat γ-globulin (passive reversed anaphylaxis). Histamine release from isolated rat peritoneal mast cells, exposed in vitro to the specific antigen, has been achieved by Perera and Mongar[4] and confirmed by Zilletti et al.[5]. So far, no information is available on the possibility of inducing an active or passive sensitization of isolated mast cells from mice. Information is also lacking on the possibility of sensitizing, actively or passively, murine neoplastic mast cells, maintained in tissue culture or in the inbred mice. The isolation of a clone (i.e. the progeny of a single cell) from a murine mastocytoma tumour possessing high histidine-decarboxylase activity, and fairly constant in endogenous amines (histamine and serotonin[6]), provided us with a system for investigating the problem of the histamine release by passive sensitization with murine neoplastic mast cells.

Methods. Furth mastocytoma cells, which were grown subcutaneously as a solid tumour in LAF_1 mice, were transplanted in tissue culture according to the method described by Fischer and Sartorelli[7]. From the original mixed population a clone of cells was obtained by means of the cloning technique by dilution[8]. This clone was maintained for one year, both in tissue culture and as an ascitic tumour in LAF_1 mice. The biochemical and biological characteristics (doubling time, 24 h; chromosome modal number, 45; histamine levels, 650 ± 54 ng/10^6 cells; serotonin levels, 750 ± 80 ng/10^6 cells) of the cells continuously grown either in culture or in the mice were the same. Since these neoplastic cells grown in mice afford higher cell population than when grown in culture, ascitic tumours were used as a source of cells throughout these experiments. The approach used to sensitize the cells passively was the production in the rabbit of an antiserum against mast cells from the same colonie. The procedure was carried out as follows: Neoplastic mast cells were obtained from the mice, washed and resuspended in ice-cold tyrode solution. Microscopic examination of small samples revealed a pure and uniform cell population. Two male albino rabbits (weighing 3 kg each) were injected endovenously with $2–3 \cdot 10^7$ cells, and subcutaneously with 2 ml of pertussis vaccine. Before the procedure for sensitization was begun, a sample of about 10 ml of blood was collected from each rabbit; the sera obtained were kept frozen until used. Booster injections of $5 \cdot 10^6–10^7$ cells were given subcutaneously every 10 days, and the schedule repeated four times. At the end of the sensitization procedure, blood was collected by cardiac puncture from each rabbit, and the sera obtained kept frozen.

Mast cells obtained from a single mouse were harvested, washed and resuspended in 6 ml of tyrode solution. A set of 5 small centrifuge tubes was prepared as follows: tube 1, 0.2 ml of tyrode + 0.8 ml of tyrode containing mast cells; tube 2, 0.2 ml of serum of rabbit No. 1 before sensitization + 0.8 ml of tyrode containing mast cells; tube 3, 0.2 ml of serum of rabbit No. 1 after sensitization + 0.8 ml of tyrode containing mast cells; tube 4, 0.2 ml of serum of rabbit No. 2 before sensitization + 0.8 ml of tyrode

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Experimental conditions[a]	Histamine retained in the cells		Histamine present in the supernatant		Histamine total		Histamine release %	P[b]
	ng/total	ng/10^6 cells	ng/total	ng/10^6 cells	ng	ng/10^6 cells		
Spontaneous release (I)	6985 ± 909	553 ± 60	2155 ± 416	166 ± 18	9140 ± 1181	720 ± 72	23 ± 2	
Effect of serum of rabbit No. 1 before sensitization (II)	7992 ± 1363	674 ± 154	3392 ± 228	307 ± 72	11,384 ± 1272	981 ± 183	31 ± 5	II vs. I < 0.2 NS
Effect of serum of rabbit No. 1 after sensitization (III)	3380 ± 909	244 ± 34	6290 ± 636	525 ± 80	9670 ± 1100	777 ± 53	67 ± 5	III vs. II < 0.01
Effect of serum of rabbit No. 2 before sensitization (IV)	7985 ± 1500	615 ± 65	3600 ± 395	302 ± 56	11,645 ± 1636	925 ± 93	31 ± 4	IV vs. I < 0.2 NS
Effect of serum of rabbit No. 2 after sensitization (V)	2057 ± 772	139 ± 26	10,450 ± 1409	786 ± 56	12,597 ± 2136	926 ± 63	85 ± 2	V vs. IV < 0.001

[a] Mean values ± S.E. of 5 replicates. [b] Calculated by means of Student's t test between the values of histamine release % in the mentioned categories.

containing mast cells; tube 5, 0.2 ml of serum of rabbit No. 2 after sensitization + 0.8 ml of tyrode containing mast cells.

The number of cells per reaction mixture was previously counted using a particle coulter counter model A. The tubes were incubated for 30 min in a metabolic shaker at 37 °C. At the end of this period the tubes were centrifuged and the supernatant fractions collected. Histamine was extracted from the packed cells by means of a slight modification of the method described by FELDBERG and PATON[9]. Histamine in the supernatant fractions and in the cells was estimated biologically on the guinea-pig ileum in the presence of atropine (10^{-7}) and methysergide (10^{-9}). The substances used were: histamine dihydrochloride (Fisher); atropine sulphate (Merck); methysergide maleate (Sandoz); and pertussis vaccine (Lilly). The values of histamine are referred as the base.

Results. The Table shows the effects of incubating mast cells with rabbit antisera against the same clone of neoplastic mast cells. In this system, the control of unspecific releasing properties of rabbit serum is afforded by the incubation of the cells with sera of the same animals obtained before sensitization. It is evident that a release of histamine significantly higher than the spontaneous release occurs only when the mast cells are incubated with the serum obtained after the sensitization procedure. In our system the spontaneous histamine release reaches values significantly higher than those observed by many authors[2,4] for spontaneous histamine release from isolated rat mast cells. The point could be explained either on the basis of a different susceptibility to lysis by mast cells from different animal species, or by virtue of the fact that our experimental conditions were definitely supramaximal, with respect to both the time of exposure to the sera (30 min) and their concentration in the incubation mixture (20%). It is considered likely that the reduction of the incubation time or of the percentage of serum concentration will lead to lower values of spontaneous histamine release.

Discussion. Clonal neoplastic murine mast cells release histamine when incubated in the presence of a rabbit antiserum against the same clone. This system could be considered a useful model for approaching the problem of histamine release in vitro by passive sensitization, for the following reasons: (a) The cell population in the ascitic fluid of mice bearing the tumour consists largely of mast cells, and the response does not differ from that of pure population grown in culture. This can avoid the procedure of isolating mast cells from other cell population normally present in the peritoneal fluid of the normal animals. (b) These cells, as progeny from a single cell, are characterized by a fairly constant histamine-forming capacity and endogenous histamine levels, as well as by the same biological characteristics (doubling time; chromosome modal number). In this way one overcomes the inconvenience of working with mast cells coming from heterogeneous cell population at different levels of evolution[10], such as one finds in those systems which make use of mast cells coming from the peritoneal fluid of normal animals.

As far as the mechanism of histamine release is concerned, studies are now in progress to elucidate if the reaction is complement-dependent, and to ascertain the nature of the antibodies involved in this reaction.

Riassunto. Mastociti neoplastici di un clono diploide, coltivati nel topo come tumore-ascite, sono stati impiegati per produrre nel coniglio un antisiero specifico verso cellule dello stesso clono. L'incubazione con l'antisiero produce liberazione di istamina da parte di mastociti dello stesso clono; l'effetto non si osserva se i mastociti sono incubati con sieri ottenuti dagli stessi animali prima della sensibilizzazione. Viene prospettato l'uso di questo modello sperimentale per sue possibili applicazioni allo studio della liberazione dell'istamina nella sensibilizzazione passiva dei mastociti.

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