The clarification of the granules after PCPA-treatment therefore supports the hypothesis that they contain melatonin and serotonin, both known for their osmium affinity[2].

The fact that this clarification is accompanied by an increase in the number of primary lysosomes can be interpreted in several ways: (a) under normal conditions, lysosomes might be involved in the discharge (secretion?) of indolamines from osmiophilic granules where they are stored[11]. Under influence of PCPA, which prevents synthesis of new amines, the stock would be heavily sollicited (clarification of the granules) and the lysosomes therefore in great demand: (b) the increase in lysosomes might be in connection with the areas of focal degenerescence found in several pinealocytes, and may be related to the formation of residual bodies; (c) the increase in lysosomes may be a non-specific reaction to PCPA[12–14].

Résumé. L'administration de PCPA, un inhibiteur de la synthèse de la sérotonine et de la mélatonine, entraîne la clarification des granules osmiophiles de l'épiphyse du rat blanc. Cette observation est en faveur de l'hypothèse selon laquelle la sérotonine et la mélatonine sont des constituants des granules osmiophiles. De plus, plusieurs hypothèses sont proposées pour expliquer l'augmentation des lysosomes primaires constatée parallèlement à la clarification des granules.

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[11] Lysosomes were shown to play a similar part in a 'secretory process' by Wetzel et al. in their study of the colloid resorption in follicular thyroid cells under the influence of TSH[12].

[12] B. K. Wetzel, S. S. Spicer and S. H. Wollman, J. Cell Biol. *25*, 593 (1965).

[13] We are greatly indebted to Dr. W. G. Forssmann for having suggested the use of PCPA, and for providing us with material from his personal experiments.

[14] This work was supported by a grant from the Fonds National Suisse de la Recherche scientifique.

A New Class of Cytostatic Folic Acid Antagonists 1-(3,4-Dichlorophenyl)-5-isopropyl-biguanide and its Boron Compounds

1-(3,4-Dichlorophenyl)-5-isopropyl-biguanide-hydrochloride (Chlorguanil, I) has been found to possess a high antimalarial activity[1,2]. With the following experimental and clinical work we were able to demonstrate the species dependence of the effect of I on hematopoiesis and tumours.

Effect on hematopoiesis. (a) Mouse, rat, rabbit and dog. The highest tolerated doses of 10 mg/kg daily i.p. or 20 mg/kg daily orally had no influence on the hematopoiesis of mice and rats. Rabbits even tolerated doses of 50 mg/kg daily orally for 20 days without developing leucopenia. The dog's hematopoiesis, too, is resistant to I[3]. (b) Guinea-pig and cat. The hematopoiesis of the guinea-pig is very sensitive to I. Guinea-pigs weighing 220–250 g were given daily doses of I in aqueous solution either by the i.p. or the oral route. Groups of 5 animals were used for each dose. The number of leucocytes was determined daily or every other day. The results of the effect of I in different doses on the number of leucocytes in the peripheral blood can be seen from the Table. A dose of 6 mg/kg daily i.p. leads within 7 days to a rapid fall of the leucocytes from a mean value of 6800 to 700 leucocytes/mm³ blood. In lower doses the leucopenia is less marked. In further investigations it was observed that granulopoiesis as well as lymphopoiesis is depressed. Schärer[3] also found that the cat showed a very sensitive response to the administration of I. (c) Man. Patients with neoplastic diseases were given I i.v. or orally. Doses of 1–2 mg/kg daily resulted in marked leucopenia and thrombocytopenia after 1–3 weeks[4].

Effect on tumours. (a) Mouse. The following transplantable tumours were used: Ehrlich carcinoma, solid form, Ehrlich ascites carcinoma and Crocker sarcoma S 180. The maximum tolerated daily doses of 10 mg/kg i.p. or 20 mg/kg orally given from the first day after implantation did not inhibit the growth of these tumours. (b) Rat. 2 rat tumours were used: Walker carcinosarcoma 256 and uterus epithelioma (Guérin) T 8. With the same dose schedule as that administered to mice, the rat tumours were not influenced. (c) Man. In preliminary clinical work[4] it was found possible to achieve some temporary objective remissions in Hodgkins' disease, lymphosarcoma, reticulosarcoma, pleural endothelioma and bronchial carcinoma. An optimal dosage schedule has not yet been worked out.

Mechanism of action. The leucopenia-inducing effect of I in guinea-pigs can be antagonized by formyl tetrahydrofolic acid (THFA)[5]. Not only the bone marrow depression but also the loss of weight and the lethality are reduced or prevented by the prophylactic use of formyl tetrahydrofolic acid. Groups of 5 guinea-pigs were given 6 mg/kg I daily i.p. without or with the addition of different doses of THFA. A dose of 6 mg/kg I daily for 6 days results in the above mentioned leucopenia, in a rapid weight loss and in the death of all animals within 6–9 days. If 12 mg/kg THFA is given i.p. daily 30 min before the administration

Effect of I on the leucocytes in the peripheral blood of guinea-pigs

Daily dose I mg/kg i.p.	Leucocytes			
	Day 1	Day 5	Day 7	Day 14
0.5	5400	3700	3900	4600
1	6500	3600	3700	3900
2	9000	3100	3400	3100
4	6800	3400	1900	No survivors
6	6800	1200	700	No survivors

Each value is the mean of 5 guinea-pigs.

[1] A. F. Crowther, F. H. S. Curd, D. G. Davey, J. A. Hendry, W. Hepworth and F. L. Rose, J. chem. Soc. 1774 (1951).

[2] F. H. S. Curd, D. G. Davey, J. A. Hendry and F. L. Rose, Br. J. Pharmac. *5*, 438 (1950).

[3] K. Schärer, unpublished results.

[4] W. Bollag, unpublished results.

[5] Formyl tetrahydrofolic acid = Leucovorin® (Lederle).

of I, the guinea-pigs show neither a leucopenia nor a weight loss and they all survive. Lower doses of THFA do not completely prevent but only reduce bone marrow depression, weight loss and lethality. A reduction of the leucopenia is still achieved, but to a lesser extent, when THFA is injected only 8 h after the administration of I. If instead of THFA the ordinary folic acid is used in doses of up to 30 mg/kg daily, the effect of I was not antagonized at all.

In experiments in vitro and in vivo the inhibition of dihydrofolate reductase activity was demonstrated by BURKARD[6]. The dihydrofolate reductase activity of guinea-pig liver is markedly inhibited. This corresponds to the sensitivity of the guinea-pig's hematopoiesis to I. On the other hand the dihydrofolate reductase activity of rat liver is not inhibited, which parallels the fact that the rat's hematopoiesis is not sensitive to I.

It is known[7] that the antimalarial biguanides are metabolized to the corresponding 4,6-diamino-1,2-dihydro-s-triazines, which are considered to be the active metabolites against plasmodia. It was first thought that those species sensitive to I would metabolize I to the corresponding diaminodihydrotriazine which is known to be a folic acid antagonist and to possess some antitumour properties[8], whereas the other species resistant to I would not metabolize I in this way.

In studying the metabolism of I in different species ESCHENHOF[9] was able to show that there is no parallelism between the metabolism of I to 1-(3,4-dichlorophenyl)-2,2-dimethyl-4,6-diamino-1,2-dihydro-s-triazine and the leucopenia or antitumour effect. Thus it may be supposed that the species dependence of this folic acid antagonist is due not to a different metabolism but to a varying sensitivity of the different dihydrofolate reductases to I.

Boron compounds. In order to obtain a more selective cytotoxic effect SPIEGELBERG and RAMUZ[10] synthesized a series of boron compounds of I, as SOLOWAY[11] had demonstrated a certain affinity of boric acid and related compounds to tumours. The majority of these compounds showed, if given orally or i.p., a similar species dependent effect on the hematopoiesis. The boric acid derivative, 4 (or 2)-[4-(3,4-Dichloroanilino)-6-(isopropylamino-1,3,5,2-triazaborin-2(5 H)-yl]-1-(3,4-dichlorophenyl)-5-isopropylbignanide, for example, induces a leucopenia similar to that of I and this effect is also antagonized by THFA.

Zusammenfassung. 1-(3,4-Dichlorphenyl)-5-isopropylbiguanid (I) besitzt cytostatische Eigenschaften, die speziesabhängig sind. I übt eine stark depressive Wirkung auf die Hämatopoese von Meerschweinchen, Katze und Mensch aus, während das Knochenmark von Maus, Ratte, Kaninchen und Hund nicht beeinflusst wird. Transplantable Tumoren von Maus und Ratte werden in ihrem Wachstum nicht gehemmt. Hingegen liessen sich beim Menschen objektive Regressionen einiger Tumorarten beobachten. Die Wirkung auf die Meerschweinchenleukopenie kann durch Formyltetrahydrofolsäure aufgehoben werden. I hemmt die Dihydrofolatreduktase der auf I empfindlichen Spezies Meerschweinchen, nicht aber das entsprechende Ferment der auf I resistenten Spezies Ratte. Der Folsäureantagonismus ist speziesabhängig, was auf eine verschiedene Empfindlichkeit der Dihydrofolatreduktasen und nicht auf einen verschiedenen Metabolismus zurückzuführen sein dürfte. Borverbindungen von I besitzen ähnliche cytostatische Eigenschaften.

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[10] H. SPIEGELBERG and H. RAMUZ, personal communication.
[11] A. H. SOLOWAY, in *Progress in Boron Chemistry* (Ed. H. STERNBERG and A. L. MCCLOSKEY; Pergamon Press, Oxford 1964), vol. 1, p. 203.

Sur le comportement d'artères «temporaires» de l'embryon de poulet explantées in vitro[1]

Dans des notes précédentes publiées dans cette revue[2-4], nous avons communiqué les résultats d'expériences portant sur un certain nombre d'artères d'embryon de poulet cultivées in vitro selon la méthode de WOLFF. On a étudié le comportement d'artères élastiques, d'artères musculaires et aussi d'une artère – l'aorte – présentant une structure élastique dans sa partie crâniale et une morphologie musculaire dans son segment caudal. Les données fournies par les cultures de l'aorte nous ont confirmé ce qu'on avait déjà observé auparavant sur les artères élastiques et musculaires, étudiées séparément.

Il nous a paru intéressant de considérer encore certaines artères «temporaires», dont la durée de vie est limitée à la période embryonnaire du poulet et qui vont disparaître au moment de l'éclosion: les artères du sac vitellin et de la membrane chorio-allantoïdienne. Les premières sont les véritables «canaux nourriciers» destinés à prendre la nourriture du jaune et à la véhiculer, par l'intermédiaire des veines, vers l'embryon. Les secondes, logées dans la membrane homonyme, réalisent par l'intermédiaire de la coquille les échanges gazeux entre l'embryon et l'extérieur.

On a étudié la genèse de ces vaisseaux temporaires sur des embryons du 5e au 20e jour. La morphologie au cours du développement est à peu près superposable dans les 2 sortes d'artères; la description que nous en donnons est donc valable, à quelques différences près, pour les vaisseaux vitellins et chorioallantoïdiens. Au 5e jour, les artères sont formées par une lame endothéliale autour de laquelle se disposent quelques couches mésenchymatiques. Ces dernières se différencient déjà au 6e jour et la morphologie des cellules rappelle les myoblastes. On distingue facilement à cet âge les artères des veines; autour des premières, les cellules mésenchymatiques sont plus abondantes et en phase plus avancée de différenciation. Cette

[1] Ces recherches ont bénéficié d'un subside du Fonds National Suisse de la Recherche scientifique et de la Fondation E. Barell de la Maison Hoffmann-La Roche SA, Bâle.
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[4] G. CONTI et B. CAPPELLI, Experientia *24*, 825 (1968).