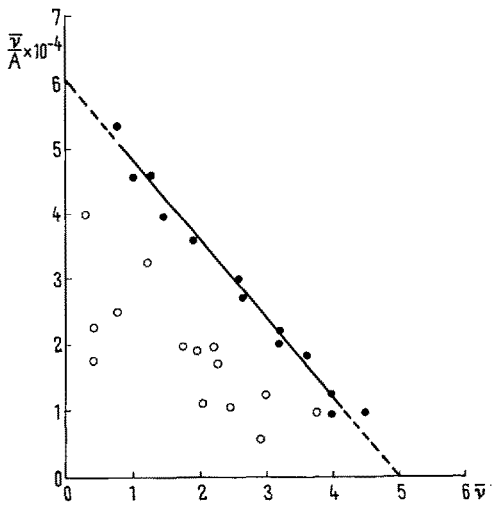
Discussion. The pharmacological significance of drug-protein interactions has been reviewed[1,7]. Diminution of drug activity from interaction of drug with protein has been reported. The data of LASSER et al.[8] suggest that potentiation of pentobarbital activity by Urokon is caused by binding of albumin allowing a high level of free or unbound pentobarbital available for central nervous activity. It has been reported that increasing the albumin concentration from 0.5% to 5% increased the amount of bound thiopental from 30% to 85%[2]. BRODIE et al.[3] demonstrated that at plasma levels of 10–50 μg, 75% of thiopental was bound to the non-diffusible component of plasma. The effect of EDTA on the binding capacity of thiopental with albumin was determined. The number of binding sites decreased to 4 and the association constant of bovine serum albumin for thiopental in the presence of EDTA decreased to 10,000. Thiopental in the presence of EDTA is less firmly bound with albumin. ONKST et al.[9] demonstrated that in the presence of calcium binding substances such as EDTA and citrate the induction time for pentobarbital narcosis was shortened[10].



Binding of thiopental by bovine serum albumin at 8°C in tris buffer, pH 7.42. (Open circles indicate the presence of EDTA in the media.)

Zusammenfassung. Die Bindungskapazität und die Anlagerungskonstante von Thiopental an Rinderalbumin wurde bei pH 7,42 in Trispuffer bei einer Temperatur von 7°C nach der Methode der Gleichgewichtsdialyse gemessen. Die Bindungskapazität betrug 5 und die Vergleichskonstante war 12000. Durch Zugabe von EDTA wurde die Bindungskapazität auf 4 reduziert; gleichzeitig verringerte sich die Anlagerungskonstante auf 10000.

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Effects of Serotonin, Anti-Serotonin and Anti-Histamine Drugs on Uracil-Mustard Intoxication

The experimental syndrome produced by acute whole body radiation, as well as the acute intoxication pattern induced by alkylating agents, can be modified by the administration of biogene amines or their antagonists. Studies with serotonin, antiserotonins and antihistaminics have yielded conflicting results[1–3].

Recently, a new alkylating agent, derived from the nitrogen mustard, uracil mustard (UM), has been introduced in the therapy of leukemias and lymphomas[4,5]. Experimental acute UM intoxication produces a pathologic pattern entirely comparable to acute intoxication by radiation or radiomimetic agents[6].

We have studied the effects of the administration of serotonin, anti-serotonin and anti-histaminic drugs on the survival time of rats treated with sublethal doses of UM.

Methods. Five groups of adult male albino rats (Wistar strain), having an average weight of 225 g, were given intraperitoneally 1.5 mg/kg 5-bis-(2-chloroethyl)-amino-uracil (UM)[7] diluted in physiological saline + 5% dimethylacetamide (as suggested by PETERING et al.[8]) to a concentration of 0.3 mg/ml.

The first group served as a control. The second group was i.p. injected, 30 min before the administration of UM, with 2.5 mg/kg 1-methyl-lysergic acid butanolamide (methysergide, UML 491)[9] at a concentration of 0.5 mg/ml. The third group was i.p. injected, 24 h after the administration of UM, with the same dose of methysergide as the previous group. The fourth group was i.p. injected, 30 min before UM, with 12.5 mg/kg 5-hydroxytryptamine creatinine sulphate (5-OHT)[10] at a concentration of 2.5 mg/ml. The fifth group was i.p. injected,

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30 min before UM, with 5 mg/kg 1-*p*-chlorophenyl-1-(2-pyridil)-3-dimethylaminopropane maleate (chlorophenpyridamine)[11] at a concentration of 2 mg/ml. The administration of the same dosages of serotonin, anti-serotonin and anti-histaminic drugs to control animals gave a survival of 100%.

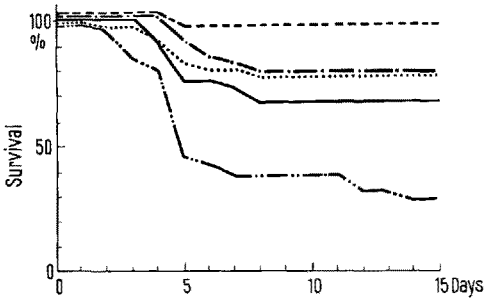
Daily observation of all animals yielded the survival rate for each group. Leucocyte counts were performed on tail blood specimens one day before and 3 and 10 days after UM treatment.

Table I

Group	Treatment	Survived/ treated rats	% survival	Probability factor (P)
1	UM alone	23/24	67.6	–
2	UM + anti-serotonin 30 min before	34/35	97.1	< 0.01
3	UM + anti-serotonin 24 h after	27/34	79.4	> 0.05
4	UM + serotonin 30 min before	10/35	28.5	< 0.01
5	UM + anti-histaminic 30 min before	23/30	76.6	> 0.05

Table II

Group	Treatment	Average leucocyte count/mm³		
		Before UM	3 days after UM	10 days after UM
1	UM alone	5.533	3.030	9.322
2	UM + anti-serotonin 30 min before	5.400	2.710	7.820
3	UM + anti-serotonin 24 h after	6.366	3.821	14.175
4	UM + serotonin 30 min before	5.766	2.433	10.657
5	UM + anti-histaminic 30 min before	6.200	2.364	9.677



Survival rates of the five rat groups treated as follows: UM alone ——, UM + anti-serotonin 30 min before – – – –, UM + anti-serotonin 24 h after –·–·–·–, UM + serotonin 30 min before –··–··–, UM + anti-histaminic 30 min before ·····.

Results. The survival data for the five groups of rats are given in Table I and in the Figure. Group 1: UM at the dosage administered permits a 67.6% survival at the end of the observation time, the highest death frequency occurring between the fourth and fifth day after treatment. Groups 2 and 3: Pre-treatment with anti-serotonin increases survival to 97.1%. The protective effect of this drug is not so evident when given 24 h after UM (79.4% survival). Group 4: Pre-treatment with serotonin increases significantly the mortality rate in rats treated with UM (28.5% survival), with highest death frequency on the 5th day after intoxication. Group 5: Pre-treatment with anti-histaminic drug does not significantly protect the rats treated with UM (76.6% survival).

White blood cell counts are reported in Table II. With UM alone, leucopenia is present 3 days after treatment. Rats surviving 10 days manifest a rebound of leucocyte count to a level exceeding pre-treatment values. Serial leucocyte counts of the other groups did not differ significantly from group 1.

Discussion. These results indicate that acute experimental UM intoxication in rats can be influenced by certain biogene amines and their antagonists as has been previously reported for nitrogen mustard and derivatives. In particular the mortality rate from UM is significantly increased after serotonin pre-treatment. Pre-treatment with anti-serotonin seems to induce a significant reversal of the lethal effect of UM. These results are in accordance with previous findings[1,2] regarding nitrogen mustard intoxication. However, when the anti-serotonin was administered 24 h after UM, no significant protection was obtained. In contrast, FIELD et al.[2] reported that the protective effect was more evident when serotonin antagonist was given after rather than before the alkylating agent. No protective effect of an anti-histaminic drug was conclusively demonstrated in these studies, in contrast to the reported[3] protection rendered against nitrogen mustard.

It is notable that although serotonin and anti-serotonin markedly altered the survival rate, the leucopenic effect of UM was not affected by either drug. A different result has been reported with nitrogen mustard[2,3]. This may be due to a real difference between the antimitotics used or merely to different experimental conditions.

In conclusion, these results confirm previous observations that certain biogene amines and their antagonists modify the body response to the administration of alkylating agents, in this case, massive doses of UM. These findings may well find useful application in the field of clinical chemotherapy.

Résumé. L'administration d'une anti-sérotonine (UML 491 ou méthysergide) réduit sensiblement la mortalité des rats soumis contemporainement à doses sublétales de uracil-moutarde (UM); l'administration de sérotonine au contraire en augmente la mortalité. L'administration de l'anti-sérotonine 24 h après l'intoxication avec UM et celle d'un anti-histaminique n'ont pas un effet protecteur statistiquement signifiant.

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