Effect of Furacin (5-nitro-2-furaldehyde semicarbazone) on Various Sulfhydryl and Non-sulfhydryl Enzymes.*† (18420)

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(Introduced by Albert G. Hogan.)

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Furacin or 5-nitro-2-furaldehyde semicarbazone inhibits the growth of a variety of gram positive and gram negative bacteria(1) and the oxidation of glucose and pyruvate in bacteria(2). In addition to Furacin, other 5-nitro-furans inhibited the oxidation of glucose(3) and pyruvate(4) by bacteria while the corresponding non-nitro analogues were completely inactive(3,4). The growth of a transplanted fibrosarcoma in mice(5,6) was also inhibited. In the following experiments the action of Furacin on various sulfhydryl and non-sulfhydryl dehydrogenases and hydrolysing enzymes from bacteria and tissues will be described.

Experimental. Experiments with isolated enzyme systems. In these experiments the effect of Furacin on purified urease (Bios), crystalline pepsin (Armour), crystalline trypsin (Armour) and papain (Nutritional Biochemicals) was observed. Urease activity was determined by the method of Folin and Wu(7) using Nesslerization. The sulfhydryl character of the enzyme has been studied by Hellerman and his group(8). The urease was exposed to 3.6 x 10^{-4} M (1:15,000) Furacin in a water bath at 37°C for periods varying from 15 minutes to an hour, along with a control containing water, both at a pH of 7.4. At the end of the exposure period, the urea substrate, buffered with Folin pyrophosphate buffer, pH 7.6, was allowed to react with the substrate for 15 minutes after which the reaction was stopped with normal hydrochloric acid. Urease activity was determined in 5 ml portions of solution containing 0.6 mg of urease and 0.4 mg of urea in all experiments. Reactivation experiments were performed with neutralised cysteine hydrochloride (Nutritional Biochemicals) using exactly the same amounts of enzyme, substrate and Furacin as above. The molar concentration of cysteine was 100 times that of Furacin. The results of these experiments are given in Table I. The observed inhibition of urease activity depends on the duration of exposure to Furacin. Reactivation with cysteine varied in the same manner. Exposure to the non-nitrated analogue of Furacin, 2-furaldehyde semicarbazone, 2.14 x 10^{-3} M (1:2,500) gave no detectable inhibition.

TABLE I. Effect of Furacin on Activity of Jack Bean Urease and Its Reactivation by Cysteine.

Initial exposure time to furacin, min.	Molarity of furacin	Mg of ammonia N per 5 ml sol.		% inhibition	% reactivation with cysteine 3.6×10^{-2} M
		Without cysteine	With cysteine		
15	3.6×10^{-4}	.046	.060	29.0	72.5
15	"	.065	.064		
30	3.6×10^{-4}	.030	.051	50.0	70.0
30	"	.060	.058		
60	3.6×10^{-4}	.023	.040	56.6	56.7
60	"	.053	.054		

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No inhibition was noted for pepsin or trypsin after an exposure to 1:10,000 Furacin for one hour, or for papain after an exposure of 2 hours to 1:7,500 Furacin. Pepsin and trypsin are classified as non-sulfhydryl enzymes (9) while Fruton and Bergmann(10) and Jaffe(11) obtained results which places papain in a controversial category. The formol titration method of Brown(12) was used in assaying the activity of these enzymes. The glass electrode was used instead of indicators for determining the end point of the formol titration.

Experiments with bacterial and tissue dehydrogenases. Using the Thunberg technic, the effect of Furacin on the formic, malic, and succinic dehydrogenases of *E. coli* was determined(2). In accordance with Gale's(13) experiments, the formic dehydrogenase of *E. coli.* is presumably non-sulfhydryl in character. This was also indicated by our experiments, since pCMB (*p*-Chloromercuribenzoate) and Mapharsen (3-amino-4-hydroxyphenyl-arsineoxide hydrochloride) in concentrations of 1.8×10^{-5} M and 1.3×10^{-4} M respectively do not affect this dehydrogenase while the malic and succinic dehydrogenases are inhibited at these levels. The α-glycerophosphate dehydrogenase of rabbit thigh muscle is non-sulfhydryl according to Hopkins and Morgan(14). No change in the reduction time of methylene blue with this enzyme from rabbit muscle was observed using Furacin (5.4×10^{-4} M).

The malic, lactic, formic and α-glycerophosphate dehydrogenases of *E. coli* were also studied aerobically in the Warburg respirometer. The method of Barron and Singer(15) was used for the malic dehydrogenase while a method similar to that used by Green(2) was used for the formic dehydrogenase. During a 2 hour period of observation, no inhibition of oxygen uptake was observed with the formic dehydrogenase in the presence of 3.6×10^{-4} M Furacin while the other dehydrogenases were inhibited. Upon the addition of 100 moles of cysteine per mole of Furacin, complete reversal of the malic inhibition was obtained while no effect was observed upon the formic dehydrogenase. The non-nitro analogue of Furacin, 2-furaldehyde semicarbazone, in a concentration of 1.4×10^{-3} M gave no detectable inhibition of the malic and formic dehydrogenases. Semicarbazide was used as a fixative in experiments with the malic and lactic dehydrogenases.

Experiments with organisms susceptible and resistant to Furacin. E. coli and *S. aureus* were rendered resistant to Furacin by transplantation in increasing amounts of the drug. The control organisms were carried through a similar series of transfers in the same media without Furacin. Several of the previously discussed enzyme systems were tested with the parent susceptible and resistant strains in the presence of Furacin and other sulfhydryl agents. The results of these experiments on some of the dehydrogenases previously mentioned using *E. coli* show that Furacin resistant organisms appear to have developed some cross resistance to sulfhydryl agents especially pCMB and Mapharsen. The other arsenicals (p-aminophenylarsineoxide, p-carboxyphenylarsineoxide) did not show this effect in all cases. The resistant strain of *E. coli* could not be differentiated from the susceptible strain by the usual cultural reactions.

In a series of experiments, the amount of Furacin and sulfhydryl inhibitor was kept at a constant level, while the weight of organisms varied from 0.75 mg to 1.5 mg per test. At the lower levels (0.75 mg) the resistant strain was relatively insensitive to Furacin and pCMB, while the susceptible strain was relatively sensitive. When both resistant and susceptible strains were increased to a higher level (1.5 mg) both strains became relatively

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TABLE II. Effect of Furacin on Various Dehydrogenases.

Enzyme source	Substrate	Methylene blue method: Conc. furacin, M	Methylene blue method: Minutes for 90% reduction	O_2 uptake method: Conc. furacin, M	O_2 uptake method: μl O_2 uptake in 60 min.
E. coli	Malate	5.4×10^{-4}	21	3.6×10^{-4}	32
"	"	—	7	—	102
"	Succinate	5.4×10^{-4}	20		
"	"	—	8		
"	Lactate			3.6×10^{-4}	237
"	"			—	514
"	Formate	5.4×10^{-4}	6	3.6×10^{-4}	129
"	"	—	6	—	133
"	α-glycero-phosphate			3.6×10^{-4}	46
"	α-glycero-phosphate			—	125
Rabbit thigh muscle	α-glycero-phosphate	5.4×10^{-4}	15		
" " "	α-glycero-phosphate	—	15		

TABLE III. Effect of *p*-Chloromercuribenzoate on Malic Dehydrogenase of a Strain of *E. coli* Susceptible and Resistant to Furacin.*

Inhibitor	Molar conc.	Minutes for 90% reduction: 0.75 mg org./test R†	0.75 mg org./test S†	1.0 mg org./test R	1.0 mg org./test S	1.25 mg org./test R	1.25 mg org./test S	1.5 mg org./test R	1.5 mg org./test S
p-Chloromercuribenzoate	3.6×10^{-5}	α‡	α	31	α	6	23	4	11
"	1.8×10^{-5}	16	38	7	16	6	12	4	6
"	9.0×10^{-6}	12	21	7	9	6	7	4	6
Furacin	5.4×10^{-4}	12	50	7	21	6	16	4	10
Control	—	12	16	7	8	6	7	4	5

* Each test was performed in a total vol. of 1.25 ml containing phosphate buffer, pH 7.2, 0.1 ml; sodium malate 0.5 M, 0.1 ml; semicarbazide 1.0 M, 0.1 ml; organisms as above 0.25 ml; methylene blue 0.001 M, 0.1 ml; distilled water or inhibitor, 0.6 ml.

† The symbols R and S represent the resistant and susceptible strains of the organism respectively.

‡ α = decolorization time greater than 1 hr.

insensitive to Furacin and pCMB. These results are given in Table III.

Urease from susceptible and resistant strains of *S. aureus* grown in 2% urea medium was tested at various levels ranging from 2.0 mg to 14.0 mg of organisms per test. Inhibition was noted by Furacin and pCMB in the susceptible strain in all cases, while the resistant strain was insensitive to both of these reagents.

Discussion. It appears from these results that Furacin inhibits some sulfhydryl enzymes. Not all sulfhydryl enzymes seem to be inhibited since Furacin had no effect on papain. However there is some controversy as to the sulfhydryl nature of this enzyme(10,11). An indiscriminate inhibition of all sulfhydryl enzymes may result in acute toxicity and may not be characteristic of a selective chemotherapeutic agent. The exact nature of sulfhydryl inhibition with Furacin remains to be determined by further experiments.

Furacin does not appear to act as an indiscriminate dehydrogenase inhibitor. The formic dehydrogenase of *E. coli* and the α-glycerophosphate dehydrogenase of rabbit thigh muscle which presumably are non-sulfhydryl in character are not inhibited by Furacin.

The experiments with Furacin susceptible and resistant strains of bacteria suggest several possible explanations. It is conceivable that in the process of the development of resistance, the overall sulfhydryl character

of the bacterial dehydrogenases may be altered. Another possibility may be a change in the permeability of the cell or the emergence of a divergent metabolic pathway. This problem can be clarified by isolating and comparing the properties of the same sulfhydryl enzymes from the parent susceptible and resistant strains.

Summary. Furacin inhibits purified jack bean meal urease as well as urease from a susceptible strain of *S. aureus*. Pepsin, trypsin and papain are not inhibited. The malic, succinic, lactic and α-glycerophosphate dehydrogenases of a susceptible strain of *E. coli* are inhibited by Furacin while the formic dehydrogenase is not inhibited. The α-glycerophosphate dehydrogenase of rabbit thigh muscle is not inhibited. Several dehydrogenases from a strain of *E. coli* resistant to Furacin show some cross resistance to p-chloromercuribenzoate and some trivalent arsenicals. A similar phenomenon was observed with the urease from a strain of *S. aureus* resistant to Furacin. It is concluded that Furacin inhibits some enzymes requiring active sulfhydryl groups.

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Right and Left Atrial Pressures in Successive Stages of Oligemic and Normovolemic Shock.* (18421)

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Using dogs under morphine and barbital anesthesia, with their chests open and under controlled lung inflation, C. J. Wiggers(1) studied the circulatory failure which developed as a result of prolonged hemorrhagic hypotension, and again after reinfusion of the withdrawn blood. It was found that the experiments could be divided into two approximately equal groups: (1) Those in which the circulatory failure developed after reinfusion as right atrial pressure declined, and (2) those in which it supervened despite the fact that right atrial pressure was maintained at or above control levels. The question arises whether the reduction in cardiac output—mainly responsible for the decline of arterial pressure after transfusion(2,3)—is accompanied by changes in left atrial pressure which are in accord or at variance with those in the right atrium. It was the purpose of this investigation to study these pressure relations by simultaneous registration of right and left atrial pressures, for it has never been proved that the beats of the two ventricles are coordinated so perfectly during oligemic and normovolemic shock that changes in filling pressure for the right ventricle are automatically accompanied by similar quantitative or even directional changes on the left side.

Methods. Dogs weighing from 10 to 15 kilos were anesthetized with morphine and sodium barbital at least 3 hours prior to any observations. The chest was opened in the midline, utilizing an electric cautery to minimize blood loss. The trachea was cannulated and respirations were maintained by means of an artificial respirator. Optical tracings of the right and left atrial and the central aortic pressures were taken on a rapidly moving photokymograph by means of calibrated Gregg manometers of adequate frequency and appropriate sensitivity. These were connected by lead tubing to rigid cannulae introduced respectively through the right jugular vein, a left pulmonary vein and the subclavian artery. A segment capsule in the air-

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