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## Effect of Furacin (5-Nitro-2-Furaldehyde Semicarbazone) on the *in vitro* Metabolism of Mammalian Tissues.* (19444)

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The effect of the antimicrobial nitrofuran, Furacin(1), on various bacterial enzyme systems has recently been described by Green (2,3), and by Asnis and Gots(4,5). Since the nitrofurans are being studied as systemic agents in the treatment of various pathological conditions, it is essential to determine the effect of such chemotherapeutic agents on the host as well as on the invading organism. Chronic oral administration of nitrofurans to laboratory animals has been associated with few histological changes other than reversible effects on seminiferous tubules and adrenal cortex(6-8). The most prominent symptom following single massive oral doses of Furacin to animals is hyper-irritability with occasional convulsions(6,8).

Studies of the effects of Furacin on tissue metabolism *in vitro* have been initiated and certain of the observed data are presented in this paper. These studies should aid in explaining some of the observed effects of Furacin in the mammalian organism (hyper-irritability, etc.). Combined with such studies as those of Green(2,3), or of Asnis and Gots(4,5), any difference in action of Furacin on mammalian and bacterial cells might be elucidated.

*Experimental.* Measurements of oxygen uptake were made with standard Warburg apparatus in an atmosphere of oxygen in Krebs-Ringer-phosphate buffer(9) pH 7.2. Anaero-

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TABLE I. Effect of Furacin on Oxygen Uptake of Various Rat Tissues Under Endogenous Conditions and with Added Glucose.

| Tissue | Substrate | Furacin conc. | No. flasks | $Qo_2$* 30 min | $Qo_2$* 1 hr | $Qo_2$* 2 hr |
|---|---|---|---|---|---|---|
| Liver | Endogenous | — | 3 | 3.2 | 3 | 2.3 |
| | " | ‡ | 3 | 2.9 | 2.7 | 1.3 |
| | Glucose .2% | — | 3 | 3.9 | 3.5 | 2.6 |
| | " " | ‡ | 6 | 4.8 | 3.2 | 1.8 |
| Kidney | Endogenous | — | 6 | 16.1 | 14.5 | 12.3 |
| | " | ‡ | 5 | 16.7 | 13.2 | 10.3 |
| | Glucose .2% | — | 3 | 16.4 | 16 | 14.3 |
| | " " | ‡ | 3 | 19.7 | 17.9 | 15.2 |
| Brain | Endogenous | — | 6 | 4.8 | 3.3 | 1.5 |
| | " | ‡ | 6 | 5.4 | 2.5 | .6 |
| | Glucose .2% | — | 3 | 11.1 | 10.1 | 9.2 |
| | " " | ‡ | 3 | 11.9 | 8.3 | 2.7 |
| Diaphragm | Endogenous | — | 3 | 4.5 | 3.4 | 3 |
| | " | ‡ | 3 | 4.5 | 1.8 | .7 |
| | Glucose .2% | — | 3 | 5.4 | 4.4 | 3.1† |
| | " " | ‡ | 3 | 5.8 | 3.3 | 1 † |
| Testis | Endogenous | — | 3 | 2.9 | 2.5 | 1.1 |
| | " | ‡ | 3 | 2.4 | .8 | .4 |
| | Glucose .2% | — | 3 | 6.2 | 5 | 3.3 |
| | " " | ‡ | 3 | 4.5 | 3.1 | 2.2 |
| Heart | Glucose .2% | — | 6 | 2.8 | 2.7 | 1.8 |
| | " " | ‡ | 6 | 2.1 | 1.5 | .6 |

* $mm^3$ $O_2$/mg dry wt tissue/hr. † Value at 1 hr 45 min. Exp. not continued for 2 hr.
‡ Furacin conc. $7 \times 10^{-4}$ M.

bic studies were made in bicarbonate buffer (9) in an atmosphere of 95% $N_2$, 5% $CO_2$. Tissue slices of rat liver, heart, kidney cortex and brain and of guinea pig kidney cortex and brain were prepared with the Stadie slicer (10) with the usual precautions. Rat testis preparations were made by teasing apart the tubules. Rat diaphragm muscle was used as strips. Furacin crystals were dissolved in the buffer-substrate medium, the concentration adjusted to the desired level after spectrophotometric determination(11), and this drug-containing medium introduced into the experimental flasks at the start of the experiment. Control flasks containing the medium without added drug were set up concurrently for comparison in all cases. Tissues and flask contents were chilled until all were prepared to facilitate simultaneous transfer to the Warburg bath. Substrate concentration is recorded in the individual graph or table for each experiment. Readings were taken at 15-minute intervals and the results calculated as the appropriate Q values for the interval over a 1½- to 3-hour period.

*Results.* The effect of Furacin upon the endogenous metabolism of the selected tissues and upon the metabolism of the same tissues in the presence of added glucose is shown in Table I. It may be seen that there is relatively little difference between the endogenous metabolism of liver and kidney and the metabolism in the presence of added glucose, indicating presence of endogenous substrate in these tissues. A carry-over of substrate by diaphragm is also indicated. The dependence of brain and testis on added substrate is shown by the rapid decrease in $Qo_2$ in the endogenous study and the maintenance of $Qo_2$ in the presence of added glucose. Marked interference in glucose metabolism of brain and testis and some interference with that of heart and diaphragm is noted in the presence of Furacin. Little or no interference by Furacin with the oxygen uptake by kidney or liver slices, either endogenous or in the presence of added glucose, is observed.

Of the tissues used, brain slices appeared the most suitable for more extended studies for the following reasons: (a) the endogenous studies confirm the observations of previous workers(12) that relatively little stored substrate is present in this tissue. This simplifies the picture when various substrates are to be

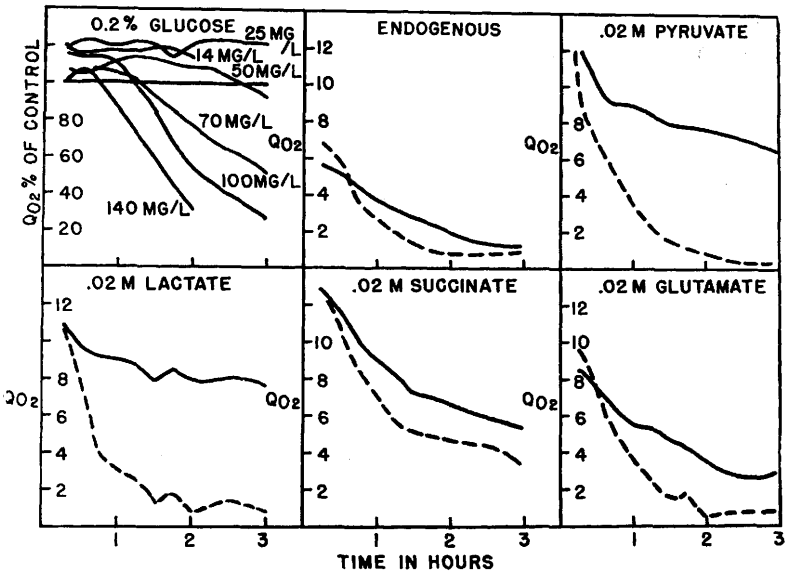


FIG. 1. Effect of Furacin on oxygen uptake of rat brain slices in various substrates. Furacin concentration = 140 mg per liter ($7 \times 10^{-4}$M) except as noted in the glucose substrate. ——— control; – – – – Furacin.

studied. (b) the $Q_{O_2}$ is high and differences due to inhibition show up readily. (c) it was of interest to continue *in vitro* studies on brain since hyperirritability and convulsions were the most obvious symptoms observed after high oral doses of Furacin.

The results of studies on the effect of various concentrations of Furacin on the metabolism of brain slices, using glucose as a substrate, are presented in Fig. 1. In this portion of the graph, the $Q_{O_2}$ values are presented in terms of per cent of control values rather than as absolute figures in order to compare directly the results from different experiments. Here it can be seen that the inhibition of oxygen uptake by Furacin decreases with decreasing concentration of the drug. At the lower levels no inhibition occurs under the conditions of the experiment. The increase in $Q_{O_2}$ observed at the lower concentrations of Furacin and during the initial periods of the higher concentrations cannot be explained at this time. Similar effects have been observed with methadone • HCl (Amidone) (13) and with sodium salicylate (14).

In attempting to locate more definitely the site of action of Furacin on carbohydrate metabolism, the effect of the drug on the respiration of brain slices in the presence of various substrates was studied. The results are shown in Fig. 1. The inhibiting effect of Furacin is most apparent in the metabolism of pyruvate and of lactate and least marked in the presence of succinate. The interference with pyruvate metabolism has also been corroborated by analytical keto-acid determinations. The following substrates were also used but did not appear to increase the respiration of rat brain slices above the endogenous level: malate, fumarate, citrate, acetate, aspartate, and glycine.

The effect of Furacin on the oxygen uptake of rat kidney cortex slices was determined under endogenous conditions and using glucose, pyruvate, lactate, succinate, glutamate, aspartate, citrate, and acetate as substrates. In the order of substrates named the $Q_{O_2}$ values at the end of the 1-hour period were as follows: 14.5 (endog.), 16.2, 18.4, 22.6, 30.4, 25.4, 20.9, 14.5, 20.5. The only medium in which significant Furacin inhibition occurred was pyruvate ($Q_{O_2}$ at 1 hr. = 75% of control and 60% at 2 hr).

Anaerobic glycolysis of glucose as measured by $CO_2$ production from bicarbonate buffer was studied, using rat brain and testis and guinea pig brain and kidney cortex slices. The control $Q^{N_2}_{CO_2}$ values observed at one hour

were as follows: rat brain 6.7, rat testis 6.3, guinea pig brain 10.7, guinea pig kidney cortex 5.4. In no case was any inhibition by Furacin observed. This has also been confirmed by analytical determination of the lactic acid produced.

*Discussion.* When glucose is used as a substrate with brain tissue, a rather long latent period (Fig. 1) occurs before the inhibitory effect of Furacin is apparent. This probably cannot be ascribed to the failure of Furacin to penetrate cell walls since, with the same type of tissue preparation (slices), no such latent period occurs in the presence of the other substrates. No appreciable interference by Furacin with anaerobic glycolysis in mammalian tissues has been found. It seems possible that the latent period in the presence of glucose may result from temporary regeneration, during glycolysis, of accessory factors necessary for certain limited oxidative pathways which may operate in the presence of Furacin. Balance studies using brain tissue with glucose as a substrate are in progress in this laboratory and the results of determinations of glucose disappearance and end-product appearance in the presence and absence of Furacin should throw further light on the mechanism of Furacin action.

The absence of inhibition in anaerobic glycolysis and the effects on aerobic metabolism in the presence of various substrates suggest that the mechanism sensitive to Furacin inhibition in the mammalian cell occurs in the aerobic utilization of pyruvate. The studies of Asnis and Gots(4) and of Green(2) on bacterial cells indicate interference by Furacin in the enzymatic dehydrogenation systems of bacteria involved in carbohydrate metabolism in both aerobic and anaerobic systems.

The present metabolic studies indicate that mammalian tissues (brain, testis) which have shown gross manifestations or histological evidence of effects from high doses of Furacin *in vivo* are tissues which are dependent on carbohydrate substrates for maintenance of *in vitro* metabolism. Interference by Furacin with the glucose metabolism of these tissues *in vitro* has been shown. It is suggested that the hyperirritability and convulsions produced by massive doses of Furacin as well as the changes in the testes may be related in a large measure to a depressed aerobic carbohydrate metabolism in the presence of the nitrofuran.

*Summary.* Investigations were carried out to determine the effect of Furacin upon the metabolism of mammalian tissue slices using Warburg manometric technics. Inhibition by Furacin was observed in the utilization of glucose by brain, testis, diaphragm, and heart under aerobic conditions. No appreciable inhibition by Furacin was observed in the oxygen uptake of kidney or liver slices either under endogenous conditions or with added glucose. No inhibition of anaerobic glycolysis by Furacin was observed in brain, testis or kidney. Studies on the aerobic utilization of various substrates by brain and kidney slices point to the inhibition by Furacin as occurring in the aerobic utilization of pyruvate.

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