TABLE 1*

Minutes	Adapted with 10 γ benzoate					Adapted with 20 γ benzoate					Adapted with 30 γ benzoate				
	No drug	1:50	1:100	1:150	1:300	No drug	1:50	1:100	1:150	1:300	No drug	1:50	1:100	1:150	1:300
60	33	6	17	23	29	137	66	93	102	124	183	57	139	139	161
90	26	3	8	15	21	71	31	44	58	62	107	36	73	83	93
120	6	−2	0	1	2	8	−2	1	3	2	54	16	35	41	45

* The percentage increases in the rates of oxidation of 5 μm of sodium benzoate by washed cells of *M. butyricum* at indicated intervals after adaptation to various concentrations of benzoate, with and without Furacin, as compared with non-adapted, drug-free cells (drug values = concentration of Furacin × 10^{-3}).

the kinetics of this process, but almost nothing is known concerning the mechanisms involved. To gain some insight into the various metabolic factors of the organism involved in this process, a series of highly specific enzyme inhibitors would be desirable. Thus, by testing the effect of each inhibitor, one could determine which is effective in delaying or stopping the process altogether.

Furacin[2] (5-nitro-2-furaldehyde semicarbazone) has recently been shown to inhibit many dehydrogenases, particularly those involved in carbohydrate metabolism (*3*). Other bacterial enzymes such as esterases, proteases, catalase, transaminase, and amino acid decarboxylase were only slightly if at all affected. The inhibition does not appear to be a drug-substrate competition, since increasing amounts of substrate do not alter the inhibition produced by a given amount of the drug. The purpose of this work was to study the effect of Furacin on the formation of the benzoate oxidase by *M. butyricum*.

The organism used was obtained from the Department of Bacteriology at Duke Hospital. It was grown in Long's synthetic medium for 3 days at 37.5° C, using 25.0 ml medium in 250-ml Erlenmeyer flasks. To prepare a suspension of the cells, the contents of each culture flask were placed in two 20 × 100-mm test tubes and centrifuged at 2,000 rpm for 15 min. Following this, the supernatant fluid was decanted, distilled water was added, and the cells were stirred vigorously with a small glass stirring rod. The contents of each test tube were then divided equally between two Hopkins tubes. Afterwards, the cells were centrifuged again for 10 min, and decanted. Buffer (0.2 *M* KH_2PO_4 with 0.2 *M* NaOH; pH 6.8) was then added, and the contents of each tube were stirred again. After a final centrifuging for 10 min, the liquid was decanted, and fresh buffer added so that 0.05 ml of packed cells was suspended in 1.0 ml. One ml of this suspension was used in each Warburg vessel, which had a fluid volume of 3.2 ml.

Cells were adapted to benzoate by incubation with 10, 20, or 30 γ, added to the main compartments of the vessels, for 45 min. Various concentrations of Furacin were also added to this compartment during the adaptation period, as shown in Table 1. Controls contained neither Furacin nor benzoate during the incubation period. At the end of this time, benzoate (5.0 μm/vessel) was added from the side arm, and the oxygen uptakes were measured.

Table 1 shows the percentage increases in O_2 uptakes as compared with controls for each set of conditions at given times. When the cells were incubated with 10 γ, the degree of adaptation was less, and the percentage inhibition by Furacin more. The highest concentration of drug gave the greatest percentage of inhibition in all cases. It will be noted that when the cells were incubated with 20 γ benzoate, the percentage increase in O_2 uptake at 60 min was 137%, and 66% with 1:50,000 Furacin. When 30 γ was employed, the percentage increase in the vessel without the drug was 183% and 57% with 1:50,000 Furacin. This is in accord with the findings of Asnis and Gots that the inhibition does not appear to be a drug-substrate competition.

[2] Thanks are due to the Eaton Laboratories for supplying a generous sample of Furacin.

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Manuscript received July 24, 1951.

The β-Glucuronidase Activity of Chemically Induced Rat Hepatoma

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Evidence has been produced by Fishman and his colleagues (*1, 2*) which indicates that in many cases cancer tissue has a higher β-glucuronidase activity than the normal tissue of origin, and it was suggested on the basis of these findings that an elevated β-glucuronidase activity may be characteristic of malignant tumors. These findings have also been taken as support for the hypothesis of Levvy and his colleagues (*3*), who have postulated a relationship between the β-glucuronidase activity of a tissue and the degree of cellular proliferation in that tissue.

One great difficulty in the interpretation of work on tumors is that of obtaining adequate controls of a

comparable cell type. The fact that in many cases the cell type of the tumor is very different from that of the tissue of origin may mean that any changes in β-glucuronidase activity only reflect a change in cell type without being related to increased cell proliferation or cancerous changes per se.

We have carried out experiments on chemically induced rat hepatoma in an endeavor to throw more light on the problem. The cell distribution in hepatoma is very similar to that of the normal liver, the main difference being in the chemical composition of the cell itself.

Stock albino rats were maintained on the diet of Griffin, Nye, Noda, and Luck (*4*), containing 0.06% *p*-dimethylaminoazobenzene (DAB) for 5 months, and a control group was fed on the same diet without DAB for the same time. An additional control group of animals was maintained on normal laboratory diet (Lever's rat cubes).

β-Glucuronidase activity was measured by a method which is essentially that of Talalay, Fishman, and Huggins (*5*). A fresh 1/100 homogenate of the tissue in water was prepared. The assay tubes contained 0.5 ml acetate buffer (0.2 *M*) pH 4.5, 0.3 ml homogenate, and 0.2 ml substrate. The final concentration of substrate was 0.001 *M*.

Desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were measured by a modification of the method of Schmidt and Thannhauser (*6*), and in addition the protein nitrogen of the livers was determined.

At the end of 5 months many tumors were present in the livers, portions of the tumor showing no necrosis being examined. In some cases, however, the livers contained many small diffuse tumors, and in these cases (which are indicated separately in the tables), the whole liver was analyzed. The results are presented in Table 1.

It will be noted that the β-glucuronidase activity of the hepatomas is much lower than that of the control livers, and those livers with diffuse tumors (Group 3) are intermediate in activity. Use has been made of the proposal of Davidson and Leslie (*7*, *8*), that in view of the suggested constancy of the DNA content of the cell nucleus for a single species, the DNA content of the tissue is a good indication of the cell number. Thus the relation of β-glucuronidase activity to DNA content indicates the β-glucuronidase per cell. In addition, since there are alterations in protein content of the tumor cell from that of the normal liver cell, β-glucuronidase activity has also been related to the protein nitrogen content of the tissue. These ratios are presented in Table 2.

It will be clearly seen that the β-glucuronidase activity/DNA ratio is greatly lowered, there being, therefore, less β-glucuronidase activity per cell in the tumor than in normal liver. It will also be noted that there is less protein in the tumor cell than in the normal liver cell, but that in spite of this the β-glucuronidase/protein ratio is lowered in the tumor cell. It is worth noting from Table 2 that while those livers with diffuse tumors (Group 3) have lost almost as much protein per cell as the hepatoma, the β-glucuronidase loss has not been so great in proportion, which suggests that in the change from liver cells to hepatoma cells not all the proteins are lost to the same degree.

In view of the reduced β-glucuronidase activity of chemically induced rat hepatoma, considerable caution must be exercised in proposing a relationship between raised β-glucuronidase activity and cancerous tissue or cell proliferation. It is more than likely that the raised β-glucuronidase activities seen in some tumors are to be related to changes in cell type and are not characteristic of the cancer tissue itself.

TABLE 1

β-GLUCURONIDASE ACTIVITY AND NUCLEIC ACID AND PROTEIN CONTENT OF RAT HEPATOMA INDUCED BY *p*-DIMETHYLAMINOAZOBENZENE AND NORMAL RAT LIVER *
(DURATION OF FEEDING 21 WEEKS)

Group	No. animals	β-Glucuronidase activity U/g† ± SE‡	DNA μgP/g ± SE	RNA μgP/g ± SE	Protein-N mg/g ± SE
1 Controls: Stock diet	10	29,300 ± 999	227 ± 9	871 ± 25	26.1 ± 0.6
2 Controls: DAB free diet	4	30,410 ± 3210	255 ± 8	833 ± 22	23.8 ± 0.9
3 Livers with diffuse tumors	6	22,100 ± 1935	371 ± 16	711 ± 26	20.4 ± 0.4
4 Hepatoma	10	15,420 ± 1070	378 ± 18	733 ± 34	20.4 ± 0.5

* All concentrations expressed on a fresh liver basis.
† β-Glucuronidase unit = 1 μg phenolphthalein liberated per hr under the conditions specified.
‡ SE = standard error of the mean.

TABLE 2

β-GLUCURONIDASE ACTIVITIES OF RAT HEPATOMA AND NORMAL RAT LIVER RELATIVE TO DNA AND PROTEIN-N CONTENTS

Group	β-Glucuronidase activity / DNA	β-Glucuronidase activity / Protein nitrogen	Protein-N / DNA	RNA / DNA
1	130	112	115	3.9
2	120	129	93	3.3
3	60	108	63	1.9
4	41	87	54	1.9

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Manuscript received July 30, 1951.

Improved Artificial Respirator for Animal Experimentation[1]

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In studies of the electrical responses of the brain to auditory stimulation in the curarized cat, it was found that the noise of the usual motor-driven respirator interfered with the experiments. Accordingly, a new respirator has been designed which, in addition to being essentially noiseless, offers other advantages:

1) The respirator adds only a small dead air space to the respiratory system.

2) There are no valves to offer resistance to air flow during passive expiration.

3) The device can be adjusted to operate at 5–40 respiratory c/min and also to shorten or extend the inspiratory phase with respect to the respiratory phase. At any given setting, the frequency of cycling and the phase relationships between inspiration and expiration are stable for long periods of operation. Frequency is not subject to change with changes of line voltage, as in the case of motor-driven devices of this kind.

4) The frequency and phase relationships can be altered during the experiment without shutting down the apparatus.

5) Electrical sparking is not involved, as with the commutator and brushes of a motor, thus making the apparatus safe for use with ether anesthesia.

6) The apparatus does not induce electrical artifacts in electroencephalographic recording.

The operating principles of the mechanical parts of the respirator are shown in Fig. 1. The mechanical parts consist of a brass cylinder with a .500-in. bore, a double-ended filleted piston machined of drill rod to give a sliding fit to the bore of the cylinder, and 2 electromagnets wound at each end of the cylinder and using the cylinder as their cores (Fig. 2). The electromagnets are alternately energized with direct current, thus drawing the piston to and fro in the cylinder. Fig. 1 shows, for the inspiratory phase, the piston drawn to the extreme of one of its excursions,

[1] Reviewed in the Veterans Administration and published with the approval of the chief medical director. The statements and conclusions published by the author are a result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

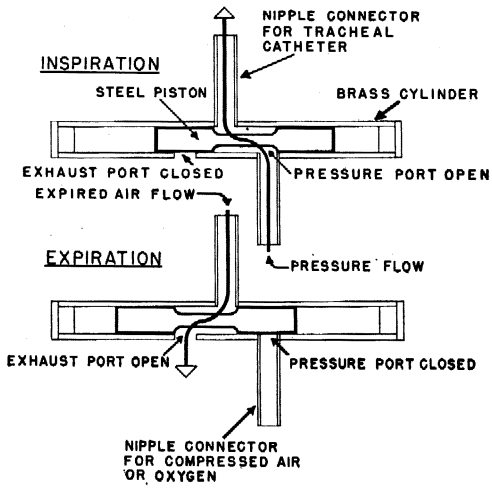

FIG. 1. Double solenoid-driven steel piston which gives free access to expired air without requiring animal's respiration to force open a valve.

allowing the compressed air or oxygen to flow through the opened pressure port, and to the animal through the tracheal connector. During this phase the expiratory port is closed. During the expiratory phase the piston is pulled to the opposite end of the cylinder, allowing free passage from the tracheal connection through the opened exhaust port, the pressure port being closed during this phase.

The cycling of the piston in the cylinder is controlled by the electronic circuit shown in Fig. 3. The electronic system is built into a small cabinet, used outside the area to which the EEG is sensitive, and is connected to the solenoids via two lines from the relay contacts and ground. These three lines may be of any length.

The functioning of the electronic system is as follows: The cycling and phasing of the system are established by the free-running multivibrator incorporating a twin-triode 6N7. Ganged potentiometers control the operating frequency. The frequency of oscillation is stabilized with the 0D3 voltage regulator. A 6J5 triode is used to operate the relay in its

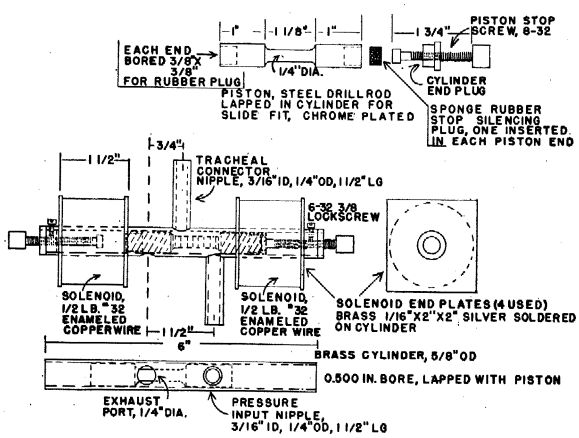

FIG. 2. Dimensions and specifications for double solenoid respirator.