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Oxidation–Reduction Potentials in the Cecal Contents of Rats and Mice (38942)

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The Oxidation–Reduction Potential (ORP) of the medium plays an important role in the cultivation of anaerobic bacteria (1). This certainly applies to the strictly anaerobic bacteria of the cecal contents of rats and mice.

It is of primary importance to attempt to obtain the same ORP in the medium as is found in the cecum of these animals. Several authors have earlier made measurements of the ORP in the cecum of rats and mice (2–6). In these cases, the measurements were made under aerobic conditions in dead rats (3), in rats which were anaesthetized (5, 6), in mice in an anaerobic environment (4), and in rats under nonspecified circumstances (2).

It is quite likely that the measurements under aerobic conditions have influenced the values for the ORP, since the ORP in the cecum is low. It is also possible that ORP values in dead animals are different than those measured in living animals. In order to investigate these possibilities, we have made continuous measurements in living anaesthetized rats and mice. Thereafter, the animals were killed and the measurement was continued. Measurements were also performed in dead rats and mice in an anaerobic environment.

The ORP values in the ceca of rats and mice were compared with the values which were found in media in which cecal bacteria were cultured and which were described in an earlier report (7).

Materials and Methods. Animals. Conventional adult Wistar rats weighing 280–320 g, and Swiss ND_2 mice weighing 26–33 g, with a Colonization Resistance Factor flora (CRF-mice) were used. These mice were the offspring of germ-free mice contaminated with the intestinal flora of a conventional mouse treated with antibiotics (8). Further germ-free Swiss mice weighing 25–33 g were used; both male and female animals were included.

Anaesthesia and killing. Ten rats, five germ-free mice, and nine CRF-mice were used for continuous ORP measurements (alive and after death) under aerobic conditions. These animals were anaesthetized by means of α-chloralose (Fluka A. G., Switzerland), 60 mg/kg body wt, in a 1% solution in distilled water (ip). This anaesthesia was supported by inhalation anaesthesia (Fluothane^R, I.C.I., England) when the electrodes were inserted. The animals were killed with an overdose of Fluothane.

Fourteen rats and ten CRF-mice were used for measurements in an anaerobic glove box which was described in a previous report (7). These animals were also killed by an overdose of Fluothane and placed in the anaerobic chamber immediately after death.

ORP measurement. A platinum and a calomel electrode were used. The method of preparation and testing was described in a previous report (9). The calomel electrode was extended by a plastic tubing containing a saturated KCL solution in a 2% agar-agar dilution. During the measurements in mice, the top of the platinum electrode was protected by a plastic cap.

A type 22 pH-meter (Radiometer, Copenhagen) was used for the recording of the ORP. The ORP was recorded continuously by means of a recorder (type BD 8, Kipp and Zn, Amsterdam) connected to the pH-meter. The ORP is given in millivolts. The Oxidation–Reduction Potentials are not converted into E_h values. The absolute reading error is taken as 7 mV.

For the insertion of the electrodes, the abdominal wall was opened and a small incision was made in the top of the cecum. Then the platinum electrode was inserted and fixed with a ligature. The calomel electrode was placed in the abdominal cavity. In the living animals, the abdominal wall and the skin were then closed by means of sutures.

The measurements were performed con-

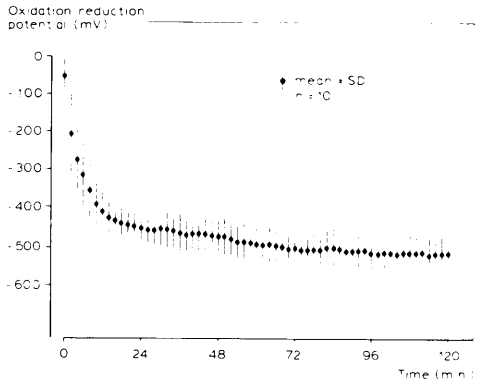

FIG. 1. Oxidation–reduction potential of the cecal contents of anaesthetized rats.

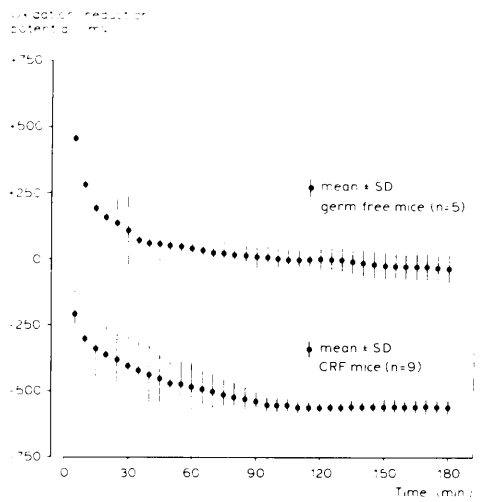

FIG. 2. Oxidation–reduction potential of the cecal contents of anaesthetized germ-free and CRF-mice.

tinuously during 120 min (rats) and 180 min (mice). After these intervals the animals were killed and the registration was continued until a constant level was reached at 4 min (rats) and 10 min (mice) after death. The cecum of the dead animals was opened for the insertion of the electrodes. In these experiments the measurements were also made about 4 min (rats) and 10 min (mice) after death.

pH measurements. Upon completion of the ORP measurement, the pH value of the cecal contents was determined by means of a calomel and a glass electrode. These measurements were made under aerobic conditions.

Results. 1. Oxidation–Reduction Potential measurement under aerobic conditions. Figures 1 and 2 show the oxidation–reduction potentials in the cecal contents of living rats and mice at the times of measurement. All ten individual curves of the rats showed an exponential decrease after insertion of the electrodes and a linear component was also observed. About 30 min after the insertion of the electrodes, the exponential decrease was almost completed and the linear decrease not yet large. From Fig. 2 it can be seen that, during roughly the first 100 min after the insertion of the electrodes, the standard deviation of the ORP in mice decreased. Thereafter, a rather constant level with only minor fluctuations in the ORP was maintained.

Table I shows the mean ORP of the rats at 30 and 120 min after the insertion of the electrodes and at 4 min after death. The ORP values for the mice at 100 and 180 min after the insertion of the electrodes and 10 min after death, are also summarized in Table I. At 120 min after the insertion of the electrodes, all ten rats show lower ORP values in comparison with the measurements made at 30 min. A significant decrease was observed during this period; the mean decrease was 66 mV (SD = 38 mV). Comparing the values 120 min after the insertion of the electrodes with values obtained 4 min after death, a further reduction in ORP is observed (sign test, $P^1 = 0.07$). The mean decrease after death is 36 mV (SD = 57 mV).

During the period of 100–180 min after the insertion of the electrodes, all of the germ-free mice showed a reduction in ORP. The decrease during this period was significant (sign test, $P = 0.06$); the mean decrease was 41 mV (SD = 17 mV). Comparing the values 180 min after insertion of the electrodes with the values 10 min after death in the germ-free group, it appeared that no significant decrease occurred. No significant decrease was found in CRF-mice between 100 and 180 min after insertion of the electrodes. Neither the sign test nor the Wilcoxon signed rank test gave a significant result ($P = 0.29$, resp. $P = 0.20$). Like the germ-free mice, the CRF mice showed no significant reduction in ORP after death.

[1] P is the significance probability.

TABLE I. OXIDATION-REDUCTION POTENTIALS IN THE CECAL CONTENTS OF RATS AND MICE (mV)

	Time (min) after insertion of the electrodes	Oxidation–reduction potential (mV) measured under aerobic conditions			measured under anaerobic conditions		
		n	Mean	SD	*n*	Mean	SD
Wistar rats	30	10	−458	45			
	120	10	−524	48			
	4 min after death	10	−560	38	14	−569	19
Germ-free mice	100	5	3	39			
	180	5	− 38	55			
	10 min after death	5	− 42	53	No measurements		
C.R.F. mice	100	9	−554	29			
	180	9	−565	26			
	10 min after death	9	−573	15	10	−573	23

TABLE II. pH VALUES IN THE CECAL CONTENTS OF RATS AND MICE.

	O.R.P. measured under aerobic conditions			O.R.P. measured under anaerobic conditions		
	n	Mean	SD	*n*	Mean	SD
Wistar rats	10	6.7	0.3	14	6.3	0.3
Germ-free mice	5	7.5	0.2	No measurements		
C.R.F. mice	9	7.2	0.2	10	7.0	0.2

2. Oxidation–reduction potential measurements under anaerobic conditions. Fourteen rats and ten CRF-mice were investigated. The ORP measurements in killed animals under anaerobic conditions are summarized in Table I. In these animals no significant difference between the ORP measurements made under aerobic and anaerobic conditions was found. The mean values for the CRF-mice were equal; the Wilcoxon test, when applied to the rats showed a significance probability, $P > 0.90$.

3. pH measurements in the cecal contents of rats and mice. The pH measurements were made under aerobic conditions after death and the results are summarized in Table II. The pH values for animals in which the ORP is measured under aerobic conditions is significantly higher, when compared to those of the animals measured under anaerobic conditions (Wilcoxon test: rats, $P = 0.04$; CRF-mice, $P = 0.05$).

Discussion. The results obtained in the present study indicate that some time is needed after insertion of the electrodes before the ORP in the cecum reaches a more or less constant level. The curve for rats suggests an exponential decrease. This is probably due to elimination of oxygen from the outside atmosphere which was introduced during the insertion of the platinum electrode. After an exponential decrease, which lasts about 30 min, a subsequent more gradual linear decrease is evident during the whole experiment. It is possible that this decrease is due to experimental manipulations. About 30 min after insertion of the electrodes the exponential decrease is almost completed and the linear decrease not large. It is likely that the ORP values measured in cecal contents about 30 min after the insertion of the electrodes come closest to the actual *in vivo* situations.

The values obtained with rats are in agreement with the results presented by Wostmann and Bruckner-Kardoss (5). The course of the curves of these authors, however, differed from ours. The curves measured by Wostmann and Bruckner-Kardoss first show a decrease and thereafter an increase, while the curves measured in this study only show a decrease. This difference is probably due to the anaesthesia procedure used. The α-chloralose used in our experiments is characterized by a long-lasting and light anaesthesia which does not influence the

vegetative nerve system and the oxygen consumption (10, 11).

The values found by Wostmann and Bruckner-Kardoss after 25 min, when the anaesthesia was the deepest and the oxygenation of the tissues the least were about the same as the values that we found after death when the oxygenation stops completely. This is in agreement with the hypothesis of Wostmann and Bruckner-Kardoss that the ORP in the cecum depends on the oxygenation of the cecal wall, among other factors. In comparison with the other authors mentioned in the introduction, the ORP values for the rats presented here, tend to be lower. This could be due to less oxygen being introduced during our experiments.

In some of our pilot experiments, the course of the curves in mice was irregular. The reason for this was found to be that the platinum electrode was forced deeply into the mucous membrane of the cecum during each cecum contraction of the anaesthetized animals. It is probable that the ORP of the cecal wall differs from that of the cecal contents and this may explain the fluctuations found. By protecting the top of the electrode, we were able to perform measurements in the contents only and the irregularities were prevented for the greater part.

The course of the curves in mice differed from the curves measured in rats. There was not a clear exponential ORP decrease in the mice; it was more gradual. After about 100 min, the ORP values for the mice reached a rather constant level. It is likely that, since mice have a smaller cecum, more time is needed for the elimination of the oxygen introduced during the insertion of the electrodes as compared with rats. During the 180 min of measurements, however, it may be that disturbing effects caused by the unnatural state of the animals had a decreasing influence on the ORP, according with the results of the rats. Since the measurements in mice take more time, this would explain why mice showed no decrease in the ORP after death in contrast to rats. We must, therefore, take into consideration the possibility that the values measured 100 min after the insertion of the electrodes are lower than the actual ORP in the cecal contents of mice.

The pH measurements for the animals studied are also given. The ORP value depends on the pH. Some authors give E_h values corrected to a pH of 7.0. The correction factor depends on the type of oxidation–reduction system (12). Since the type of oxidation–reduction system in the cecal contents is not known, we did not correct our values for the pH.

The ORP values found in the present study are within the range of those which were found earlier in the artificial media in use for the cultivation of strict anaerobes from the cecum of mice (7). The present study indicates that the ORP is at the same level *in vivo* and *in vitro*; consequently it is likely that the cultivation of anaerobic bacteria *in vitro* as described in an earlier report (7) is not negatively influenced by the ORP level.

Summary. The oxidation–reduction potential (ORP) in the cecal contents of conventional rats, germ-free mice, and mice with a Colonization Resistance Factor flora (CRF-mice) was investigated. By using animals that were anaesthetized for a longer period of time, we attempted to eliminate the disturbing influence of oxygen. In addition, measurements were made under anaerobic conditions. For the rats, the ORP values reached a more or less constant level after about 30 min following the insertion of the electrodes. The mean ORP at that time was −458 mV (SD = 45 mV). The mean ORP values for the mice showed a more gradual reduction than was found in the rats. The curves leveled off at about 100 min following the insertion of the electrodes. The mean ORP values 100 min after electrode insertion were: germ-free mice, + 3 mV (SD = 39 mV); CRF-mice, −554 mV (SD = 29 mV). In rats, the ORP value decreased after death; no decrease was observed in mice. No difference was found in the values obtained when measuring under anaerobic or aerobic conditions after death.

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