

Oxidation-Reduction Potentials in Cecal Contents of Germfree and Conventional Rats.* (30979)

BERNARD S. WOSTMANN AND EDITH BRUCKNER-KARDOSS

Lobund Laboratory, University of Notre Dame, Notre Dame, Ind.

The absence of a viable flora in germfree animals leads to a number of deviations from the normal morphological and functional characteristics of the gastrointestinal tract. In germfree rats, mice, rabbits, and guinea pigs the cecum is enlarged (1), while the wall of the small intestine is thinner and carries smaller villi with less absorptive surface (2). Permeability of the tract as expressed by passive absorption is greater in the germfree rat (3). Proteases and carbohydrases occur in higher concentrations (4,5). These differences from "normal" indicate that in the germfree animal an entirely different set of biological parameters has been established, which will profoundly influence the experimental results obtained with this animal.

Phillips and Wolfe pointed out that oxidation-reduction potential in the cecal contents of guinea pigs in the presence of a viable microbial flora was more negative than under germfree conditions (6), but no details of experimental conditions were given. Recent studies in our laboratory have indicated differences in Fe and Cu metabolism between germfree and conventional rats (7), and in Fe metabolism between germfree and conventional rabbits (8). These results prompted us to reinvestigate the effect of the intestinal microflora on the oxidation-reduction potential of intestinal contents. The cecum was chosen for this study on account of obvious technical advantages, and because in the conventional rat the bacterial population density in the average small intestine is only one-tenth of that found in the cecum (10^9 and 10^{10} per gram of contents respectively).[†] Observations made in the cecum could therefore be expected to approximate conditions in most of the small intestine.

Materials and methods. Germfree and con-

ventional male Lobund rats of Wistar origin were used in all experiments. The animals were approximately 3 months old and had been maintained on sterilized commercial diet 5010C.[‡]

Germfree rats were removed from the germfree isolator just before the start of experiment. All animals were anesthetized with 35 mg pentobarbital sodium per kg body weight. A 1-inch incision was made in the abdominal wall and the basal portion of the cecum with the ileocecal valve was exposed. Two small incisions were made in the wall of the small and large intestine about 5 mm from their junction with the cecum. A Beckman glass electrode (#39290) was inserted into the cecum *via* the incision in the large intestine, and the corresponding calomel electrode (#39270) was inserted *via* the incision in the ileum. After the pH of the cecal contents was determined, the glass electrode was replaced by a Beckman Pt electrode (#39281). The incision was then closed as far as possible with a large hemostat. Oxidation-reduction potential measurements were made at 5 minute intervals for the next 30 minutes, followed by 2 readings at 10 minute intervals. Subsequently the Pt electrode was removed, the glass electrode was reinserted and a final pH reading was taken.

In another series germfree rats were inoculated with a mixed cecal flora taken from conventional animals. To this end, the animals were removed from the germfree environment and placed in a fiberglass hood where surgical sterility was maintained. The abdominal region was painted with iodine and the abdominal cavity was opened under light anesthesia, using sterile surgical techniques. The large and small intestine were ligated approximately one inch from their junctions with the cecum, leaving the blood supply intact. Half of the animals then re-

* This investigation was supported specifically by Office of Naval Research, Grant Nonr-1623(13) and also generally by University of Notre Dame.

[†] Personal communications, M. Wagner.

[‡] Pelleted rat diet manufactured by Ralston Purina Co., St. Louis, Mo.

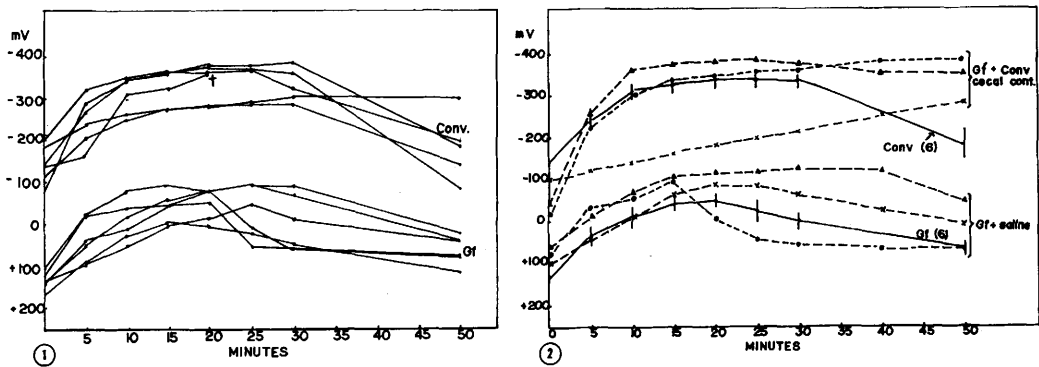


FIG. 1. Oxidation-reduction potential in cecal contents of germfree (GF) and conventional (Conv.) rats under pentobarbital anesthesia.

FIG. 2. Oxidation-reduction potential in cecal contents of germfree rats inoculated intracecally with cecal contents of conventional rats or with sterile saline 4 hr prior to determination. Solid lines depict averages and SDM values of results given in Fig. 1.

ceived 0.5 ml of a 1:5 dilution of conventional cecal contents in saline injected into the cecum. The other animals received an injection of sterile saline only and served as controls. The incision was closed with wound clips and each animal was placed in a pre-sterilized glass jar which was closed with glass fiber filter material. All animals remained in the jars for 4 hours. They were then removed from the jars and pH values and oxidation-reduction potentials were determined as described above.

All oxidation-reduction potentials were converted to E_h values at pH 7.0, using the average of the two pH readings and $-\Delta E_h/\Delta \text{pH} = 0.0591$ (9). The standard potential of the saturated calomel electrode was taken to be 236 mV.

Results. The oxidation-reduction potentials of the cecal contents of germfree and conventional rats measured under pentobarbital anesthesia are depicted in Fig. 1. Average values at the beginning of the period,

during the period of deepest anesthesia, and at the end of the experiment when most animals showed signs of recovery, are given in Table I, which also shows average pH values of the cecal contents. In both experimental groups the oxidation-reduction potentials became increasingly more negative during the first 15 minutes, then stabilized, to become more positive again towards the end of the experimental period. During the entire period the average potentials shown by the conventional rats were 250-300 mV more negative than those found in the germfree animals. The pH values of the cecal contents of the conventional rats were approximately 1 unit lower than in their germfree counterparts (6.6 ± 0.1 and 7.5 ± 0.1 , respectively).

When germfree rats were inoculated intracecally with cecal contents of conventional animals, oxidation-reduction potentials measured 4 hours after inoculation resembled the values found in conventional rats (Fig. 2). Inoculation with sterile saline produced values

TABLE I. Oxidation-Reduction Potentials and pH Values of Cecal Contents of Germfree and Conventional Rats Under Pentobarbital Anesthesia. E_h values calculated at pH 7.0. Values \pm SDM in mV.

Status	No. of rats	Oxidation-reduction potential			Avg pH
		5 min	25 min	50 min	5 to 50 min
Germfree	6	+ 35 \pm 22	- 24 \pm 25	+ 60 \pm 15	7.5 \pm .1
Conventional	6	-246 \pm 24	-339 \pm 21	-183 \pm 36	6.6 \pm .1
Germfree & cecal contents*	3	-206 \pm 40	-314 \pm 57	-341 \pm 30	6.1 \pm .3
Germfree & saline†	3	- 5 \pm 20	- 53 \pm 50	+ 4 \pm 33	7.1 \pm .1

* Inoculated intracecally with conventional cecal contents 4 hr prior to experiment.

† Inoculated intracecally with sterile saline.

that fell within the range found for germ-free animals. Four hours post inoculation ex-germfree animals treated with a conventional intestinal microflora had a lower pH in the cecal contents than the saline treated controls (6.1 ± 0.3 and 7.1 ± 0.1 , respectively). In both groups pH values were approximately 0.5 unit lower than under established germ-free and conventional conditions (Table I).

Discussion. Results given in Fig. 1 and in Table I show that throughout the experimental period the E_h values in the cecal contents of germfree rats were 250 to 300 mV more positive than the values measured in conventional animals. The data appear to confirm the observation of Phillips and Wolfe in guinea pigs(6). This difference in E_h values does not represent a systemic adjustment of the germfree animal to life without an intestinal microflora, but only reflects the absence of such a flora which normally creates a more reducing environment by its metabolic activity. This is demonstrated by Fig. 2, which shows that within hours after inoculation of the germfree cecum with a mixed flora obtained from conventional rats the E_h values reach the more negative level characteristic for conventional animals.

It is difficult to estimate the true E_h value of cecal contents of the intact rat. The values obtained directly after insertion of the electrodes would tend to be on the positive side because of the opening of the peritoneal cavity to the outside atmosphere. Values obtained halfway through the experimental period, when anesthesia reaches its greatest depth, would tend to be on the negative side because of the generally reduced blood circulation(10), leading to reduced oxygenation of the intestinal tissue. Under those conditions E_h values measured in conventional rats, with both metabolizing intestinal tissue and an actively metabolizing intestinal microflora, may reach values which almost approach the theoretically possible negative limit of -420 mV at pH 7.0. Values measured at the end of the experimental period, when the animals start to recover from pentobarbital anesthesia, would seem to come closest to the actual values in the intact rat. In the germfree rats these values fall within the range given by

Clark(9) for aerobically metabolizing cells and tissues (0 to $+200$ mV at pH 7.0). Under conventional conditions E_h values fall in the range of the E_m values measured for the DPN and FAD systems(8). Although to the authors' knowledge no further data on oxidation-reduction potentials of germfree animals are available, the more positive values found in germfree rats and in germfree guinea pigs would seem to indicate a general phenomenon in germfree mammals.

The pH values measured in the absence of an intestinal microflora closely resemble those of the circulating blood. In conventional rats these values average 1 pH unit lower, presumably indicating the formation of acid metabolites by the microflora.

The difference in oxidation-reduction potential which exists between cecal contents, and presumably between the contents of the small intestine, of germfree and conventional animals, will affect the status of a number of nutrients. As the ferrous form appears to facilitate absorption of the Fe complex (11), the difference in intestinal E_h values between germfree and conventional animals could cause certain forms of Fe to be more readily available to the conventional animal. This would explain the hypochromic anemia found in germfree, but not in conventional rabbits on certain diets(8).

Summary. Oxidation-reduction potentials in cecal contents of germfree rats were 250 to 300 mV more positive than in the contents of conventional animals. Treatment of germfree rats with cecal contents from conventional rats showed that 4 hours later these animals demonstrated oxidation-reduction values comparable to those found in conventional rats. The more positive oxidation-reduction potential thus appears directly related to the absence of an intestinal microflora.

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Received November 16, 1965. P.S.E.B.M., 1966, v121.

Influence of Growth Hormone (GH) on Content of GH in the Pituitaries of Normal Rats. (30980)

L. KRULICH* AND S. M. MCCANN†

Departments of Physiology, University of Pennsylvania, Philadelphia, and University of Texas Southwestern Medical School, Dallas

By contrast with the well known feedback mechanisms which operate between the hypothalamo-hypophyseal system and its respective target glands, the possibility of so-called auto-feedback mechanisms in which the level of a hormone would directly influence its own secretion from a gland is not well documented. That such a mechanism may exist in the case of insulin secretion was deduced from observations that insulin administration could decrease the beta cell granulation and insulin content of the pancreas(1,2).

In the case of anterior pituitary hormones, a similar mechanism may be operative for adrenocorticotrophin (ACTH) because chronic injections of ACTH were observed to decrease its content in the pituitary of adrenalectomized rats(3) and to elevate its content in the gland of normal animals(4). More recently, Szontágh and Uhlarik(5) showed that chronic administration of human chorionic gonadotrophin would reduce the hypophyseal content of LH. In the present paper, data are presented on the effects of administration of exogenous GH on its content in the pituitary which indicate that in this case also a similar auto-feedback mechanism may be postulated.

Methods. Two series of experiments were

performed. In the first series, normal adult male rats of the Sherman strain (body weight 260-290 g with an average of 275 g) were injected with bovine GH (NIH, GH B7).‡ One group received a single dose of GH 24 hours before sacrifice, the second group was injected daily for 5 days and killed on the 6th day (24 hours after the last injection) and the third group was given GH for 10 consecutive days and sacrificed on the 11th day. Five animals were in each group. All the animals received 6 mg of GH per dose, that is about 2.2 mg/100 g/day. GH was given subcutaneously dissolved in physiological saline.

The second experiment was performed in the same manner, except that smaller animals were used (body weight 165-195 g with an average of 178 g) and the dose of GH was elevated to 10 mg per dose, so that the average dose per day was 5.6 mg of GH per 100 g body weight. Instead of a single control group, 3 groups of normal animals were used, which were injected with physiological saline following the same pattern as in the case of GH injections.

Both groups of experimental animals were killed by stunning followed by exsanguination, and immediately thereafter the whole pituitaries were removed. Pooled pituitary extracts from a given group of rats were

* Present address: Praha II, Albertov 5, Fysiologický ústav, Czechoslovakia.

† This study was supported by Nat. Inst. Health Grant AM-01236-09.

‡ The bovine GH (NIH, GH B7) was supplied by Nat. Inst. Health.