

FOUNDATION 2000 GRANT REPORT

Title: Well-Being of Rabbits Immunized with Freund's Complete Adjuvant

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This report summarizes the results of a study undertaken to demonstrate that Freund's Complete Adjuvant may be used humanely for polyclonal antibody production in rabbits. 32 young female New Zealand White rabbits were divided into three groups. The first group of 12 rabbits received a mixture of Freund's Complete Adjuvant and keyhole limpet hemacyanin (KLH) for the initial immunization. For the booster immunization, Freund's Incomplete Adjuvant and KLH were administered. The second group of 12 rabbits received saline and KLH for both the initial and booster immunizations. All booster immunizations were performed 28 days after the initial immunization. The control group of eight rabbits was not immunized.

For the immunization procedure, the rabbits were sedated with a combination of 0.5 mg/kg acepromazine and 0.01 mg/kg buprenorphine given subcutaneously. The hair was shaved from the dorsum and the skin was cleaned using three povidone-iodine soap washes followed by three alcohol rinses. The immunogens were prepared using aseptic technique. Each rabbit was administered 0.1 ml subcutaneously in four sites and 0.02-0.03 ml intradermally in 20-30 locations for a total immunogen volume of 1.0 ml.

Body weight, body temperature, and degree of induration at the injection sites were determined on days 0 (baseline), 3, 6, 11, 14, 21, 28, 33, and 38. Blood was collected on days 0, 6, 11, 28, 33, and 38 to determine white blood cell count and corticosterone level. Blood was collected following sedation of the rabbits with the acepromazine/buprenorphine dosages listed above. Finally, food consumption and activity were determined daily on days -7 through 0 (baseline), days 0 through 9, and days 28 through 38. Food consumption was calculated by measuring the amount of food eaten by the rabbits over a 24-hour period. Activity was determined by placing an activity monitor on a harness worn by the rabbits. The rabbits were acclimated to the harnesses during the seven day baseline phase of the study. The rabbits were necropsied on day 38 and skin, lung, liver, axillary lymph node, and kidney were evaluated microscopically by a veterinary pathologist.

There were no significant differences between the three groups for body weight, body temperature, white blood cell count, corticosterone level, food consumption, and activity. There was a significantly greater degree of induration in the rabbits that received Freund's Adjuvant. Similar pathologic changes were seen in all three groups and consisted of pulmonary microgranulomas, subacute portal hepatitis, and nephropathy and mineral deposition in the kidney. These lesions were considered background changes as they were seen in all groups. Granulomatous inflammation was seen in the axillary lymph nodes and skin from rabbits immunized with Freund's Adjuvant. These changes were expected since adjuvants attract macrophages to the immunized areas in the skin, from where, they travel to the axillary lymph nodes.

The results of this study support the hypothesis that there were no physiologic or behavioral changes induced in the rabbits immunized with Freund's Adjuvant. In

conclusion, we believe Freund's Adjuvant may be used humanely for polyclonal antibody production in rabbits if certain criteria for its use are followed. These criteria include: 1) aseptic preparation of the immunogen, 2) surgical scrub of the immunization sites, 3) sedation of the rabbits, and 4) small volumes of immunogen injected into multiple sites. By following these procedures, Freund's Adjuvant may be used humanely for antibody production.

Title: The effects of environmental enrichment on murine immune responses

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The experiments carried out in this proposal were designed to determine if the practice of environmental enrichment influences immune responses in laboratory mice. We chose to look at this question in a commonly used strain of laboratory mice, BALB/c, and with a commonly employed model system, the immune response to bovine albumin. In a series of five experiments, we measured cytokine and antibody responses and thymocyte numbers to assess various functions and parameters of the immune system. In a final experiment, we determined the effect of stress on the ability of these mice to respond to infection with a complex pathogen, murine malaria.

There were two striking findings from these studies. The first was that the number of litters born to mice in enriched cages was dramatically fewer than the number born to those in unenriched cages. The number of mice per litter was not different between the groups, nor were reports of poor mothering between the groups. This unexpected result prevented us from carrying out one of our objectives (to compare the effects of change on immune response, rather than just the effects of enrichment) because we had insufficient numbers of mice for comparative experiments. We should note that while this finding is highly statistically significant, only four males from each group were being compared.

The second finding is that, consistent with our original observation, the thymuses of the mice that had undergone the enrichment process were dramatically smaller. The cells lost were primarily immature thymocytes. This loss was only seen in female mice, whereas the thymuses of male mice were uniformly small relative to the female group. We believe that the most likely reason for thymocyte loss is the stress-induced production of endogenous glucocorticoids. We should note that in the two experiments in which we weighed the mice, the mice that remained in an unenriched environment exhibited a trend toward being heavier, but this did not quite reach significance in either experiment ($p = .12$ in a one tailed T test). These findings are important when considering the housing of mice used for studies of thymic function.

Despite the relatively dramatic effect of enrichment on breeding and thymocyte numbers, immune responses, as assessed by cytokine and antibody production, were not significantly affected. These experiments were carried out in a single mouse strain with a

single type of immunogen, so it is not clear if they can be generalized. However in this commonly used system, it appears that environmental enrichment would not have a significant impact upon the immune responses measured.

Title: Surveillance of *Coxiella burnetii* Infection in Sheep: Detection of Shedders by DNA Amplification Using Polymerase Chain Reaction

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RESEARCH OBJECTIVES

The objectives of this study were to:

evaluate the efficacy (sensitivity and specificity) of DNA amplification by polymerase chain reaction (PCR) for detection of *C. burnetii* in various clinical specimens (blood, feces, urine, amniotic fluid, colostrum, milk, and placental tissue); and determine if DNA amplification by PCR is an appropriate method to screen for potential shedders among sheep used for biomedical research (compared to serological tests for detection of *C. burnetii* in infected sheep).

Project Progress Report

Q fever is a zoonosis of worldwide distribution, caused by the rickettsia *C. burnetii*, and is an occupational hazard for personnel in the livestock industry, meat-processing plants, veterinary medical profession, and laboratories working with the causative agent. Although infection among domestic livestock is often unapparent, it may constitute a health risk for individuals working with infected livestock and for animal care personnel and researchers utilizing pregnant sheep in biomedical animal research facilities. Biomedical researchers, utilizing sheep in facilities where exposure may occur to contaminated material such as dust, urine, feces, amniotic fluid, placentas, and newborn lambs, are at particular risk. To protect animals and humans from being infected, vaccines have been developed; however, studies have shown that the vaccines in ewes were able to reduce but not eliminate the shedding of rickettsiae. Thus, the availability of more sensitive and specific tests to assess the “infective” or shedder status of individual animals is essential for controlling the shedding of the organism by sheep used in biomedical research facilities. In this regard, we suggested that DNA amplification by PCR could be used to identify infective sheep that are shedding *C. burnetii*. Such identification would have significant application in the identification of and minimization of risk associated with contact with pregnant sheep. To this end, we infected pregnant and non-pregnant ewes with *C. burnetii* (nine mile strain) and tested subsequent clinical specimens by PCR assay. Serologic status of sheep was evaluated and correlated with PCR results.

Ewes were obtained from Q fever negative vendor sources and determined to be free of *C. burnetii* infection at the initiation of the study (based on serological and PCR screening assays). Ewe reproductive cycles were synchronized and time-dated breedings were performed. Pregnancies (12 time-dated pregnant ewes) were confirmed with

ultrasound after gestation day 25. Three groups of four time-dated pregnant ewes and one non-pregnant ewe were housed in separate animal biosafety level 3 isolation facilities (ABSL-3 practices; ABSL-3 safety equipment; ABSL-3 facilities) prior to challenge. Time-dated pregnant ewes were inoculated with 4.2×10^4 plaque forming units of *C. burnetii* nine mile strain phase I (RSA493/307GP/1TC/2EP) via subcutaneous injection of 1 ml saline suspension (10,000 infective mouse doses) and intranasal inhalation of 1 ml saline suspension (10,000 infective mouse doses) at 65 (3 ewes), 100 (3 ewes), and 135 (3 ewes) days of gestation. Three non-pregnant ewes (one in each group) were also challenged by the same procedure. Three time-dated pregnant ewes served as non-challenged controls (one in each group). After challenge, using ABSL-3 practices; a) blood, fecal, and nasal swab samples were collected weekly; b) urine samples were collected periodically; c) at the time of delivery, lamb's blood, amniotic fluid, colostrum, and placenta samples were collected; and d) after delivery, blood, fecal, milk, nasal swab, and urine samples were collected weekly.

Briefly, seroconversion was determined by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence assay (IFA) for *C. burnetii* phase I and phase II. All seronegative ewes seroconverted between one and four weeks after challenge inoculations. *C. burnetii* was detected in various biological samples by using a nested PCR technique to increase the sensitivity of the assay by directly reamplifying the product from a primary PCR (using flanking primers) with a second PCR (using internal primers). *C. burnetii* DNA was repeatedly amplified from amniotic fluid, blood, and placental tissues. Colostrum, fecal, milk, nasal swab, and urine samples are still being analyzed with variable results. Four ewes aborted and one ewe lambed one dead and one weak lamb. Severe necrotizing placentitis was present in five ewes, and the organism was detected by nested PCR. Furthermore (and of great relevance), some of the ewes that had normal lambs and placentas still shed the organism at the time of parturition (as *C. burnetii* was detected by nested PCR). This emphasizes the fact that ewes may be asymptomatic and shed the organism despite being seropositive. These ewes may be an important source of infection to other animals and humans that are in contact with them.

In summary, there is a tremendous need (from a public health perspective) for the development of improved diagnostic methods to detect *C. burnetii* in animals and clinical specimens. Currently, the serological diagnosis of infections with *C. burnetii* is based on IFA and ELISA techniques. These serological tests are adequate for giving an indication of prior exposure to *C. burnetii* but are inadequate for identifying infected animals and the potential for shedding infectious organisms. New diagnostic methodology, such as nested PCR detection of *C. burnetii*, which is directed at detecting *C. burnetii* DNA in a variety of clinical specimens and at gathering information regarding potential shedding of infectious rickettsial agents, provides a valuable and potentially much more useful approach. In view of its sensitivity and broad applicability, the PCR detection of *C. burnetii* has significant applications in assessing the shedding potential of livestock used in medical research and in minimizing the potential risk of Q fever outbreaks in research facilities.

Title: Efficacy of Oral Buprenorphine in Rats

Kristal, Mark B., University at Buffalo

Executive Summary:

a) The dose of buprenorphine commonly recommended for postoperative analgesia (0.5 mg/kg, eaten in flavored gelatin) is ineffective in producing analgesia in a pain threshold assay (Martin, et al., 2001).

b) An effective PO dose (comparable to 0.05 mg/kg, SC) is 5 mg/kg, but is too aversive (probably bitter) for rats to eat (Martin, et al., 2001).

c) The PO dose of buprenorphine that is sufficient to produce a significant elevation of pain threshold is difficult to disguise sufficiently so that all rats will eat the substance. A mixture of peanut butter and sugar was investigated, but the rats could still tell when it contained buprenorphine, and some would not eat it.

d) The effective analgesic PO dose of buprenorphine and the effective analgesic SC dose of buprenorphine both produce some gastrointestinal distress in rats, as evidenced by the induction of a conditioned taste aversion.

e) Our finding that the commonly accepted clinical method for inducing postoperative analgesia in rats (feeding 0.5 mg/kg buprenorphine in flavored gelatin) is ineffective in elevating pain threshold (Martin, et al., 2001) was not due to problems in drug preparation.

f) Our finding that the commonly accepted clinical method for inducing postoperative analgesia in rats (feeding 0.5 mg/kg buprenorphine in flavored gelatin) is ineffective in elevating pain threshold (Martin, et al., 2001), was not due to a strain difference in the rats tested.

g) The effective analgesic SC and PO doses of buprenorphine both reliably produce pica in Sprague-Dawley rats, but not in Long-Evans rats.

h) One publication has already resulted from this research (Martin, et al., 2001, and two others are in preparation. The results have already been presented at the AALAS Tribranch Symposium - *Good Care for Research Animals – Alleviation of Pain and Distress*, (Philadelphia, 6/01), and the AALAS Niagara Falls Conference (6/01).

Published study:

Martin, L.B.E., Thompson, A.C., Martin, T., Kristal, M.B. 2001. Analgesic efficacy of orally administered buprenorphine in rats. *Comp. Med.* **51**:43-48.

In that study we demonstrated that the conventional post-surgical analgesic, 0.5 mg/kg buprenorphine dissolved in flavored gelatin, is insufficient, by a factor of 10, to produce an increase in pain threshold, as measured as tail-flick latency in Long-Evans rats. When the dose was increased to 5 mg/kg PO, which, when given by orogastric infusion, the effect was comparable to that of an SC dose of 0.05 mg/kg. However, the rats would no longer eat the flavored gelatin that contained that much buprenorphine (concentration of 1.25 mg/ml); it was apparently too bitter. An additional finding was that in our pain-threshold assay technique, the analgesic effect of buprenorphine, when produced by the appropriate doses, did not last as long as commonly expected (more than 4 hr, but less than 8 hr), but was consistent with durations reported in the literature.

As yet unpublished studies:

1. *A substitute for flavored gelatin.* Because rats would not eat flavored gelatin containing 1.25 mg/ml buprenorphine (to produce a PO dose of 5 mg/kg), we attempted to identify a food substrate in which 5mg/ml buprenorphine could be orally administered. The problem with diluting the concentration of buprenorphine in flavored gelatin was that the volume necessary for them to obtain the required dose became too large to be practicable. We investigated the utility of a mixture of peanut butter and sugar (PB&S).
 - a. In pilot studies, PB&S proved to be palatable to the rats and relatively easy to use. N = 3 rats readily consumed PB&S containing buprenorphine at a 5 mg/ml concentration (the higher concentration was necessary because we wanted to reduce the volume of substance to be eaten).
 - b. Recipe (made per rat):
 - i. Smucker's Natural Peanut Butter™ (pour the oil off into a separate container for later use).
 - ii. Take 2g peanut butter and add back 0.5ml of the oil - mix well
 - iii. Sprinkle 0.5g sucrose over the top of the mixture
 - iv. Add 1 ml/kg distilled H₂O or buprenorphine solution (conc: 5 mg/ml) and mix well
 - c. Habituate all the rats to the PB&S one time before testing
2. *Efficacy of buprenorphine in peanut butter.* We compared the analgesic efficacy of the buprenorphine-in-PB&S mixture with that of buprenorphine solution administered by orogastric infusion.
 - a. Buprenorphine eaten in PB&S appears to be as effective as orogastric infusion in inducing analgesia. (Fig. 1)
 - b. In contrast to the pilot study, not all rats ate the buprenorphine in this study. (Fig. 2)

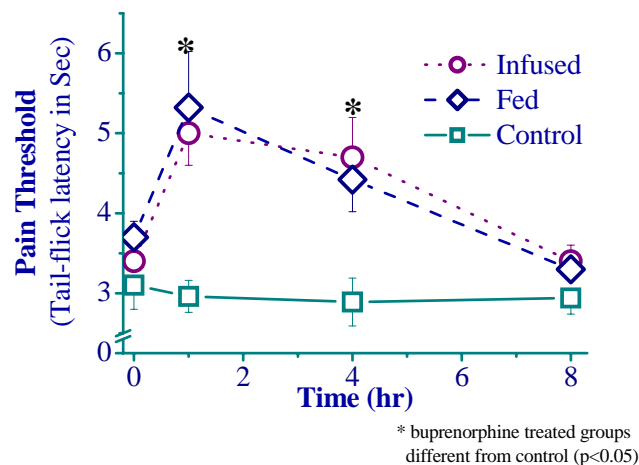


Fig. 1

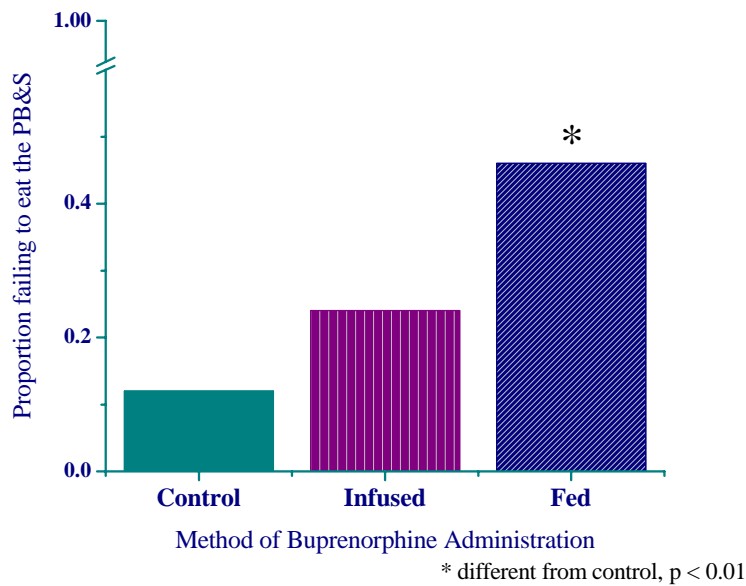


Fig. 2

3. *Can rats detect buprenorphine in peanut butter?* The fact that not all the rats would eat the buprenorphine-in-PB&S mixture led us to ask whether rats could still discriminate the buprenorphine when it was mixed into PB&S.
 - a. We used a two-dish test in which one contained plain PB&S and the other contained PB&S containing buprenorphine (5 mg/ml concentration). Rats had been habituated to the PB&S one time. Each dish contained $\sim 1/2$ the volume of PB&S used in the original study.
 - b. We recorded which dish they approached/sniffed first, which they ate from first, and whether or not they ate the contents of both dishes.
 - c. Rats were given 30 min (15 min longer than the test above) to finish eating, however, rats were *never* seen eating after 10 min. The majority of rats finished eating both dishes in 10 min. Those rats that did not eat the contents of both dishes did completely finish the content of one dish in 10 min and then left the other dish untouched for the remaining time.
 - d. We expected that if rats could not discriminate the buprenorphine, they would be equally likely to eat the plain PB&S or the PB&S+buprenorphine first; and equally likely to consume both the PB&S and the PB&S+buprenorphine entirely. We also expected that they would approach each dish randomly if there was no difference in the sight or smell of these two substances ($\sim 1/2$ would approach the PB&S dish first and $\sim 1/2$ would approach the PB&S+buprenorphine first).
 - e. The results suggest that rats can discriminate PB&S+buprenorphine from plain PB&S in that rats were significantly less likely to eat the PB&S+buprenorphine (Fig. 3).

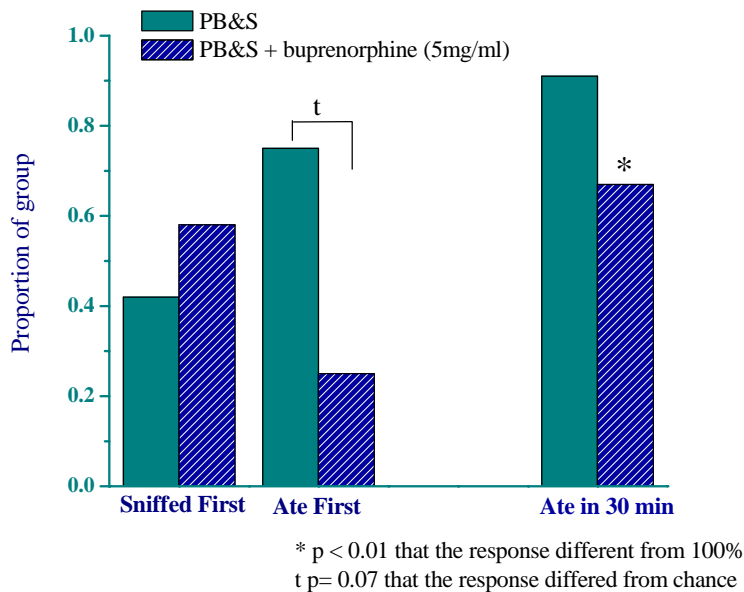


Fig. 3

4. *Does buprenorphine cause gastrointestinal distress?* Buprenorphine (like morphine) often produces nausea in humans. We attempted to determine whether the effective analgesic dose of buprenorphine (both the PO and SC doses) produced gastrointestinal distress in rats, a fact that might influence the decision as to whether buprenorphine is useful clinically for postoperative analgesia. To determine this, we tested whether buprenorphine, at doses that induce a significant increase in pain threshold (5mg/kg, PO and 0.05mg/kg SC), can induce a conditioned taste aversion (TAC). TAC is often used to determine if drugs or foodstuff induces gastrointestinal distress (nausea, diarrhea, vomiting, cramping, etc.). The general idea is that rats will avoid substances that have previously made them ill, particularly if the substance was novel at the first presentation when they became ill, and even if the substance was gustatorily and olfactorily attractive. In the TAC test, an attractive novel substance is paired with a drug (Conditioning Day). Days later, that novel substance is presented again and the willingness of the rat to ingest that substance is recorded (Testing Day). If the rat is reluctant to eat/drink the novel substance on the Testing Day, that observation is taken as evidence that the drug produced gastrointestinal distress, and consequently, that a conditioned aversion developed toward that novel substance. In our study, the novel substance was grape juice (50% Welch's unsweetened white grape juice – mixed in distilled H₂O). Rats were maintained during the 2 weeks before the test on a water-restricted diet (30 min ad lib/day) so that they would be highly motivated to drink during both the conditioning and testing periods. On the Conditioning Day, plain water was replaced with grape-juice-flavored water, and 30 min after the end of the drinking period, rats were injected (0.05mg/kg, SC) or infused (5 mg/kg, PO) with buprenorphine. Two days later, rats were offered the grape-juice-flavored water again. Fluid intake was measure throughout.

- a. All rats drank less grape juice than plain water on the Conditioning Day. This is a natural behavior of rats and other animals toward novel ingestible substances (mild neophobia). On the Testing Day, we found that **control rats** drank significantly more grape juice than they did on the Conditioning Day and that their intake of grape juice on the Testing Day was not significantly different from their baseline water intake. Therefore, as expected, when no aversive gastrointestinal experience occurred, rats increased the intake of grape juice, after the first exposure, to the baseline level of fluid intake. This also indicates that although the grape juice is not so attractive that it leads to overdrinking, it was attractive enough so that they did not avoid it on its own merits.
- b. In contrast, buprenorphine, regardless of route of administration, induced TAC. On the Testing Day, neither group of buprenorphine-treated rats increased their intake of grape juice from the Conditioning Day. This resulted in a significant group difference (Fig. 4) on the Testing Day between the PO buprenorphine group and the control group (the SC buprenorphine was between the other two groups.)
- c. This result suggests that buprenorphine induces some gastrointestinal distress at the recommended analgesic doses.

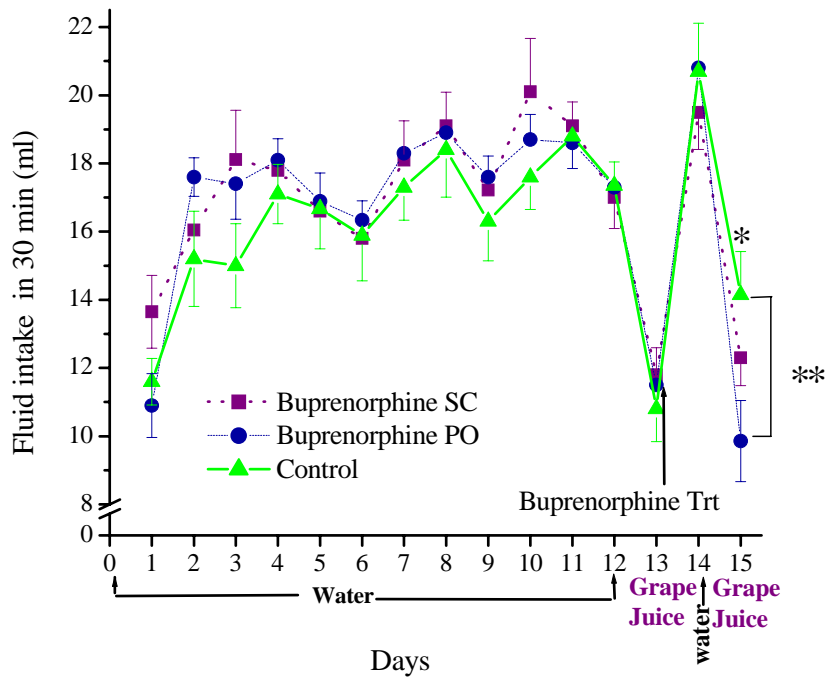


Fig. 4

5. *Strain comparison.* The initial reports suggesting the effectiveness of 0.5 mg/kg buprenorphine eaten in flavored gelatin were based on observations in albino rats. Our study (Martin, et al., 2001) criticizing that procedure was based on data collected in Long-Evans (hooded) rats. We just completed a study comparing the analgesic efficacy of PO (5mg/kg and 0.5mg/kg) and SC (0.05mg/kg) buprenorphine in Sprague-Dawley rats, in an attempt to determine whether our results with Long-Evans rats generalizes to albinos.

The results with Sprague-Dawley rats were comparable to the previously reported results observed in the LE rats (Fig. 5).

- a. For comparison, see Fig. 1 for data from LE rats collected under the same time course as the SD rats.
- b. Small strain differences in the maximal effect occurred, and these are consistent with the reports in the literature (Fig. 6). However, such strain differences cannot account for our observation that a PO dose 10x higher than the recommended 0.5 mg/kg buprenorphine is needed to induce a level of analgesia comparable to that produced by 0.05 mg/kg, SC .

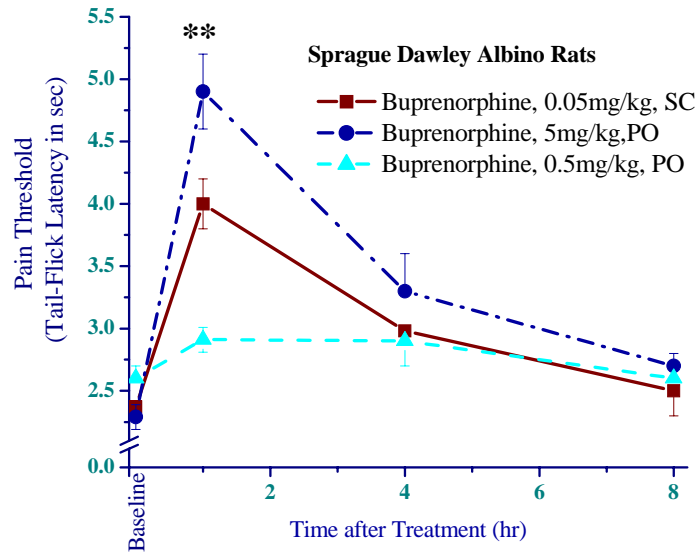
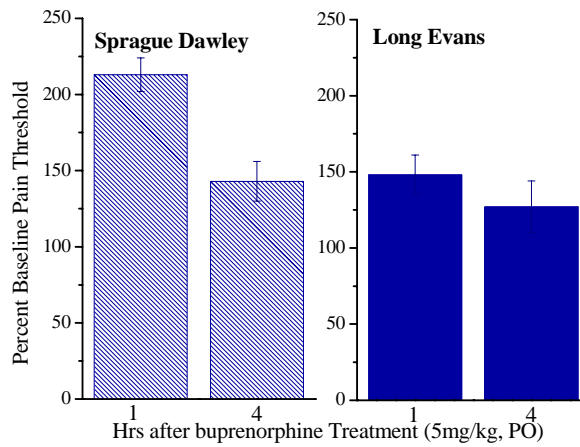


Fig. 5



Data from Fig 5 and Fig 1 - regraphed.

Fig. 6

6. *Drug preparation.* To counter a criticism we received that our results in the Martin, et al. (2001) paper might have been due to an inappropriate method of drug preparation, we compared the analgesic efficacy of buprenorphine prepared in our lab from powder (buprenorphine HCL, Sigma) with that of commercially available injectable buprenorphine (Buprenex®, Norwich Eaton). In this comparison, buprenorphine was prepared in the two ways that corresponded to the methods of preparation we used in our published experiments to make the higher PO dose of buprenorphine (5 mg/kg at 5 mg/ml), which is a complicated preparation method, and the SC dose of buprenorphine (0.05mg/kg at .05 mg/ml), which is a more straightforward preparation method. The higher PO dose (5 mg/kg) was then diluted to a concentration of 0.05 mg/ml to make it comparable to that of the SC dose. Buprenex® comes in a .3 mg/ml concentration, so it was diluted with distilled water to .05 mg/ml. The different preparation methods were tested at the SC dose of 0.05 mg/kg for equivalent analgesic efficacy.

a. No statistically significant differences between methods of preparation were observed (Fig. 7).

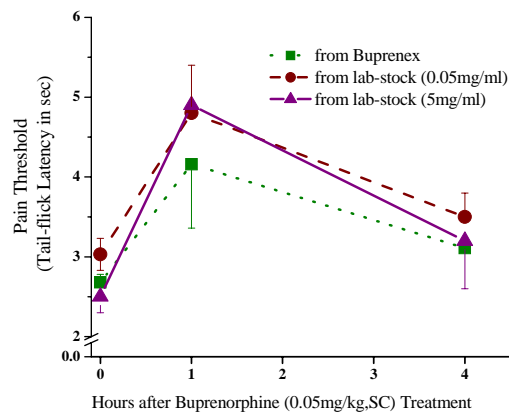


Fig. 7

7. *Strain differences in pica.* An incidental observation we made that we are investigating more systematically is that in Sprague-Dawley rats, buprenorphine, at all the doses we used, and with both PO and SC routes of administration, produced pica (ingestion of bedding and feces) in virtually every rat. On the other hand, none of the Long-Evans rats we tested showed pica.

Conclusion:

We found that oral buprenorphine used for postoperative analgesia, in the recommended dose and method of administration (eaten as .5 mg/kg dissolved in flavored gelatin), does not produce a significant elevation of pain threshold. This finding cannot be attributed to minor methodological considerations. Furthermore, when the amount of buprenorphine in flavored gelatin is increased to a clinically effective dose (5 mg/kg), comparable to the analgesic potency of 0.05 mg/kg SC buprenorphine, the rats refuse to eat it. Perhaps there are vehicles for buprenorphine that will disguise the taste sufficiently, but these will be difficult to find. The decision to continue to look for such a

vehicle for buprenorphine, though, should be tempered by our finding that the effective PO and SC doses of buprenorphine produce gastrointestinal distress, and by our finding of major strain differences in pica. It may be the case that buprenorphine should not be the drug of choice for postoperative analgesia. The one issue that remains to be tested, when additional funds are acquired, is whether the effective dose of buprenorphine is significantly lower in rats experiencing prolonged pain (pain test), than it is in the assays we used (pain-threshold test), which involve otherwise healthy rats.

Title: Development of Long-Acting Analgesics for Relief of Postoperative Pain in Rodents

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Laboratory animal veterinarians are often faced with difficult decisions choosing pain medication, especially for rodents. Some drugs such as oxymorphone and morphine are very effective at relieving pain in laboratory animals. However, the short dosing intervals make them difficult to administer, since animals can become aggressive with repeated injections. Therefore, less effective drugs with longer dosing intervals such as buprenorphine, have been used more frequently in rats and mice.

Studies using liposome-encapsulated oxymorphone hydrochloride and morphine sulfate indicate that a single injection of these preparations are effective at relieving pain in rats. Liposomes are small artificial membranes that can be engineered to contain and then release many different types of drugs. Liposome encapsulation of highly effective pain medication can be used to make preparations that provide sufficient analgesia using one subcutaneous injection given just before surgery.

Oxymorphone hydrochloride was encapsulated into liposomes using dehydration-rehydration methods. Morphine sulfate was encapsulated using shaker-batch techniques. Both methods can readily be adapted for large-scale production. Using these technologies, liposome-encapsulated oxymorphone can be produced that is released *in vitro* for 5 days. Liposome-encapsulated morphine can be produced that is effective for either 3 or 6 days depending on the chemical composition of the liposome. One subcutaneous injection of liposome-encapsulated oxymorphone or morphine was effective at relieving pain associated with nerve damage in rats. Foot withdrawal time in response to a heat stimulus is commonly used as a measure of analgesic efficacy. Foot withdrawal times were measured for rats before sciatic ligation surgery, then daily for 7 days after the surgery. Foot withdrawal times in rats given blank liposomes dropped significantly by day 4 after surgery (mean=7.1 sec.) and the effect was maximal at day 7 after surgery (mean=5.1 sec.). Foot withdrawal times in rats given LE morphine sulfate remained stable at day 4 (mean=9.8 sec.) and day 7 (mean=10.4 sec.). Foot withdrawal latencies in rats given LE oxymorphone were stable at 4 days after surgery (mean=9.6 sec.), but increased at day 7 (mean=12.2 sec.).

The liposome-encapsulated oxymorphone preparation was also tested for its efficacy against post-operative pain in rats that had intestinal surgery. This experiment was done in rats with gut resection or transection surgery. The degree of discomfort was measured using behavioral observations. Behavioral data was analyzed using two-way analysis of variance with replication. One injection of 1.2 mg/kg liposome-encapsulated oxymorphone was as effective as injections of 0.3 mg/kg of the standard pharmaceutical preparation given every 4 hours for relief of post-operative pain in rats ($p=0.18$). The rats that were given liposome-encapsulated oxymorphone prior to intestinal resection also had significantly higher body weights at the end of the experiment than animals that were given the standard pharmaceutical preparation of oxymorphone. The mean body weight change from day 0-7 of rats given 1.2 mg/kg LE oxymorphone was +9.4 g. The mean body weight change from day 0-7 of rats given standard oxymorphone was -3.6 g ($p<0.05$).

Additional experiments are planned to evaluate the effectiveness of liposome-encapsulated oxymorphone at relieving post-operative pain in mice. A nonprofit corporation is being formed to produce and distribute liposome-encapsulated pain medications for use in laboratory animals.

Project Title: Spacing behavior and social preferences of laboratory rabbits

Dr. Joy Mench

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Although laboratory rabbits are social and very active, they are often housed individually in cages and provided with minimal physical enrichment. Individually caged rabbits show higher levels of abnormal behavior than rabbits group housed in floor pens (Gunn, D. and Morton, D.B., 1995, *Applied Animal Behaviour Science* 45:277), although whether this is due to lack of social companionship, the lack of physical/structural enrichment, or both, has not been determined. Caged rabbits may also develop osteoporosis and other bone abnormalities (Stauffacher, M., 1992, *Animal Welfare* 1:105) due to the restriction of movement in the cage. However, group housing has disadvantages in terms of aggression and hygiene. We therefore examined pair housing in cages as a possible alternative to individual or group housing, and evaluated rabbits' preferences for species-appropriate enrichments. Since space in cages is limited, we also investigated the relative importance to rabbits of social interaction and physical enrichment.

In our first study, we determined the effect of pairing in cages on abnormal behavior and locomotion. We also examined whether aggression was a problem in paired rabbits. At weaning, female New Zealand White rabbits were housed either individually in single cages (N=5 rabbits) or in non-littermate pairs (N=10 rabbits) in double-wide cages (measuring 122 x 76 x 41 cm). Behaviors were sampled 3 times per day: at dawn and dusk when rabbits are most active, and during the middle of the light period. Each rabbit was observed five days a week for 15 minutes/day until 24 weeks of age. We found that

abnormal behaviors (bar-biting, floor-chewing, stereotyped digging) increased in individually housed rabbits but not in paired rabbits. Pair-housed rabbits locomoted more than individually housed rabbits. Aggression (biting, chasing, snapping, lunging) among pairs was observed infrequently, did not increase during the study, and resulted in only occasional minor injuries except in one pair that had to be separated due to persistent bite wounds.

In our second study, we characterized the use of various enrichment objects by the rabbits. Rabbits from the first study, as well as similarly aged SPF rabbits from a commercial source, were housed either individually (N=6) or in non-littermate pairs (N=12 rabbits) in floor pens measuring 1.22 x 2.44 x 0.62 m. Each rabbit was observed for 20 minutes/day for six days, during the morning, midday, and evening. Pens contained plastic nestboxes, PVC tunnels, hay cubes, and “bunny blocks” (Bio Serve, Frenchtown, NJ). Overall, rabbits used the enrichments 15% of the time. The nestbox was used more than other enrichment objects; rabbits tended to sit on top of, rather than inside of, the nest boxes. The tunnel was the next most preferred object. Social status did not influence enrichment use, which indicates that enrichments were not monopolized by the more dominant rabbit in the pair.

In our final study, we conducted a “consumer-demand” based preference test in order to determine the relative importance of social and physical enrichments to the rabbits. Rabbits from the second study were used in this third study. The test rabbit was placed into a large central area of the test apparatus, from which it could choose from one of four resources (a familiar rabbit, an unfamiliar rabbit, food, or enrichments in the form of a nestbox and tunnel) by using the appropriate tunnel. Each tunnel contained two one-way doors entering the resource pen and two one-way doors exiting the resource pen. The first entry door to each resource was gradually weighted such that, over the course of the 14-day trial, the doors became progressively heavier, which meant that the rabbits had to “work” to obtain access to the enrichments or to other rabbits. Exit doors remained unweighted. Each door was wired to a computer that monitored whether the door was open or closed, and subsequently determined the movements of the test rabbit. Due to potential problems with aggression, a horizontally-barred PVC barrier separated the test and stimulus rabbits. We found that rabbits showed a stronger preference for the unfamiliar conspecific than for the familiar conspecific or enrichments, both in terms of how much weight they pushed in order to gain access to each resource and the time spent with each resource, although there were individual differences, with some rabbits preferring their pair-mates to the unfamiliar rabbit.

SUMMARY

In these studies, we compared the behavior of female rabbits housed singly or in pairs in conventional cages, evaluated the use of biologically relevant enrichments by rabbits housed in floor pens, and assessed the relative importance of social and physical enrichments to rabbits using a consumer-demand based preference test. Overall, we found that pair housing in cages was successful in reducing the development of abnormal behaviors like bar-biting and stereotyped digging, and led to significant aggression in only one of the five pairs. Additionally, the increased cage space in the double-sized cages allowed more locomotion, probably an important factor in helping to reduce or

prevent osteoporosis. When they were housed in floor pens, rabbits used the physical enrichments about 15% of the time. They used the nestbox the most and usually sat on top, suggesting that a shelf in a cage might be a relevant enrichment. The tunnel was also used regularly. In the preference test, however, the rabbits were willing to work harder to gain access to social companions than to the tunnel or nestbox. Their strongest preference was generally for an unfamiliar rabbit rather than for their pairmate. However, this should not be taken to mean that contact with a pairmate is unimportant, nor suggest that mixing unfamiliar rabbits is desirable. It does, however, emphasize the importance of novelty in an enrichment program. Taken together, these studies indicate the importance of social enrichment for rabbits. A social companion is highly preferred and can provide novelty that cannot be provided by static physical enrichments, and the presence of a social companion is effective in reducing the development of abnormal behaviors.

Title: Determination of Shedding Frequency of B Virus in Pair-Housed Rhesus Macaques

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Non-human primate herpesviruses establish and maintain a lifelong persistent infection in immunocompetent hosts in the absence of clinical signs of disease. A fundamental issue for understanding the natural history of non-human primate herpesviruses is whether the viruses are maintained in a truly latent state or one characterized by a low level of chronic expression. To address this issue, a real-time PCR assay was developed to quantify Cercopithecine herpesvirus type 1 (B virus) DNA in mucosal fluids of rhesus macaques. This assay was rapid, sensitive (10 genome copies) and specific for B virus obtained from multiple species of macaques. The shedding profile of B virus was compared to another endemic herpesvirus, rhesus cytomegalovirus (RhCMV), in colony-reared monkeys. Mucosal swabs or saliva samples were taken daily from two groups of seropositive monkeys undergoing either a stressful relocation (group 1) or daily chair restraint (group 2). B virus DNA was detected in mucosal fluids from four animals relocated during the breeding season (group 1) but not from 10 animals moved at other times of the year. No B virus DNA was detected in any group 2 monkey. In contrast, RhCMV DNA was detected in the majority of animals of both groups 1 and 2. Detection of B virus DNA shedding is a relatively rare event associated with the breeding season, while RhCMV DNA is persistently detected in mucosal fluids of most monkeys. For full details please see Huff, J., R. Eberle, J. Capitanio, S. S. Zhou, and **P. A. Barry**. 2003. Development and Application of a Real-Time PCR Assay for Detection of B Virus and Rhesus Cytomegalovirus in Rhesus Macaques. *J. Genl. Virol.* **84**:83-92.