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Laparotomic Approach for Collecting Serial Hepatic Biopsies in Rats (*Rattus norvegicus*) and Mice (*Mus musculus*)

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Researchers often consult with laboratory animal veterinarians for suggestions on how to improve their protocols. We assisted a researcher in performing serial liver biopsies in rats (*Rattus norvegicus*) to assess the transport of iron on a cellular level. We developed a novel collection approach that used laparotomy through a midline abdominal incision and disposable biopsy punches to obtain liver samples at 3 different times at various intervals. We hypothesized the survival of the subjects undergoing the multiple survival procedures would be independent of the weight loss or gain sustained throughout the study. Although 2 rats died during the study, the results were statistically significant with regard to survival when comparing the Belgrade rats to the Sprague Dawley rats and Swiss Webster mice and were independent of the weight loss or gain incurred during the study. We also performed a pilot study in mice (*Mus musculus*), using the same method as in the rats, with equivalent results. Our study showed the survival of rodents that underwent multiple laparotomies and liver biopsies was independent of the weight gain or loss throughout the study.

Laboratory animal veterinarians often are called on to assist principal investigators with their research projects and procedures. Because of our experiences and backgrounds, we can refine procedures to make them more efficient, more productive, and less intrusive. On one such occasion, a researcher asked our department to assist with the collection of liver biopsies from Belgrade rats (*Rattus norvegicus*) after they had undergone oral iron loading and treatment with a binding agent. Our review of the available literature revealed a lack of data that supported a single, standard method for collecting multiple liver biopsies in rodents, regardless of whether the technique was invasive or noninvasive. The current pilot study presents a method for collecting a series of 3 liver biopsies from rats and mice that results in minimal hemorrhage, adhesion formation, and scarring. The method uses an open laparotomy technique and a disposable biopsy punch.

Our major concerns when we considered performing multiple major surgeries in a single animal were that we adhere to all regulatory documents, maintain the wellbeing of the animal subjects, and provide an adequate scientific justification for the procedures. According to the *Guide for the Care and Use of Laboratory Animals*,[5] multiple major surgeries can be performed on a single animal only when they are an essential component of a research protocol, scientifically justified by the investigator, or necessary for clinical reasons.[5] We ensured that this need was an integral part of the researcher's protocol and that there was scientific justification for performing multiple procedures on the same subject. Our researchers had a limited number of subjects in their colonies that could be included in their studies, and the biopsies were used to show the effects of diet over time on the liver of each subject. When performing multiple, major surgeries on a single animal, appropriate measures to support the animal's wellbeing must be implemented throughout the study.

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Standard technique for collecting liver samples from rodents requires either laparotomy or euthanasia.[4,10,12] Although considered invasive due to the need to incise the skin and musculature of the abdominal body wall to access the abdominal cavity, laparotomies offer better visualization of the tissue and permit the acquisition of larger and more diagnostically useful samples than those obtained by percutaneous needle aspirates.[1,2] In recent years, the use of percutaneous needle biopsies to obtain samples from the liver has increased in favor, but samples collected in this manner may be too small for diagnostic purposes, can be damaged during removal from the needle, and may originate from an inappropriate site.[1,2,6,8,13] Percutaneous needle biopsies can be obtained by using a blind procedure or with the aid of specialized techniques, such as ultrasonography. With a blind technique, the tissue is not visualized, and samples might be acquired from the sites of previous biopsies or from organs other than those intended to be sampled. Ultrasonic assistance is desirable but may not be available at many institutions. Even though laparotomy is invasive, it provides sufficient visualization of the liver so that tissue can be harvested from the most appropriate site, and the use of a biopsy punch to collect hepatic tissue samples provides adequate sample with minimal damage to the remaining liver lobes. Previously described techniques often encouraged the removal of an entire liver lobe or the use of cautery to achieve hemostasis.[1,10,14] However both partial hepatectomy and electrocautery decrease the quantity and quality of liver tissue available for subsequent biopsy procedures.

The purpose of the current study was to establish a laparotomy-based approach for collecting liver samples of sufficient size and quality that could be performed multiple times on the same animal and that did not negatively affect the health of the rodents or the histologic quality of the collected tissue. Our results demonstrate that the use of a disposable biopsy punch to obtain liver biopsies in rats (*Rattus norvegicus*) and mice (*Mus musculus*) causes minimal damage to the liver, provides adequate tissue specimens to perform diagnostic testing, and does not negatively affect the health of the animal when per-

formed multiple times, thereby enabling researchers to reduce the number of subjects required in their studies.

Materials and Methods

Animals. This study was conducted in the AAALAC-accredited Laboratory Animal Facilities of the Comparative Medicine Department at the University at Buffalo (State University of New York). Swiss Webster mice (n = 6; male, 2; female, 4; age, 25 to 111 d; weight, 10 to 40 g; bred inhouse), Sprague–Dawley rats (n = 2, both male; age, 199 to 247 d; weight, 500 to 520 g; bred inhouse), and Belgrade rats (n = 9; male, 3; female, 6; age, 428 to 563 d; weight, 234 to 452 g; bred inhouse) were singly housed in conventional cages with shavings for bedding (Aspen Shavings, Nepco, Warrensburg, NY) and in rooms with a 12:12-h light:dark cycle, in which the humidity (30% to 70%), temperature (68 to 79 °F), and air exchanges (10 to 15 hourly) were within the ranges set forth in the *Guide for the Care and Use of Laboratory Animals* for these species.[5] All of the indicated animals were free of parasites and negative for viral and bacterial pathogens. The Swiss Webster mice and Sprague–Dawley rats had unrestricted access to standard rodent chow (Teklad 2018, Harlan Laboratories, Dublin, VA). The Belgrade rats were provided, an iron-base powdered diet *ad libitum* (Teklad 8604, Harlan Laboratories) and a nylon bone (catalog no. 3203 or 3580, BioServ, Frenchtown, NJ) to maintain appropriate incisor length, except for the 14-d period between the first and second procedures, when they were fed a carbonyl iron diet to achieve the iron loading in the liver necessary to emulate the previous study.[6,7] All of the rodents were provided with drinking water *ad libitum*. Cages were changed once weekly, and animals were checked 3 times daily. Sentinel testing was performed twice annually to ensure that mice were free of epizootic diarrhea of infant mice virus, Theiler murine encephalomyelitis virus, minute virus of mice, *Mycoplasma pulmonis*, mouse parvovirus types 1 through 5, pneumonia virus of mice, Sendai virus, cilia-associated respiratory bacillus, lymphocytic choriomeningitis virus, ectromelia virus, mouse adenovirus types 1 and 2, mouse cytomegalovirus, polyoma virus, and reovirus 3 and that rats were free of cilia-associated respiratory bacillus, H1 virus, Kilham rat virus, *Mycoplasma pulmonis*, rat minute virus, rat parvovirus, parvovirus NS1, pneumonia virus of mice, rat coronavirus (sialodacryoadenitis virus), Sendai virus, lymphocytic choriomeningitis virus, and reovirus 3.

Surgery. All of the animals in this study received preoperative buprenorphine (rats, 0.03–0.05 mg/kg SC; mice, 0.05 mg/kg SC). Anesthesia was induced with 3% to 5% isoflurane and maintained with 1% to 2% isoflurane during surgery. All of the animals were placed in dorsal recumbency and prepared aseptically for surgery. Liver biopsies were obtained by using a disposable biopsy punch (catalog no. 33-31-P/25 [2 mm, mice] or 33-32-P/25 [3 mm, rats], Integra Life Sciences, Plainsboro, NJ; Figure 1). At the completion of surgery, each animal received a NSAID (carprofen 5 mg/kg SC) and was returned to its home cage. During the postoperative period and for the remainder of the study, all of the animals were maintained under the same conditions as outlined in the previous section.

All of the animals received oral NSAID medication (carprofen 5 mg/kg) once daily for 2 d after each procedure. Each animal was permitted 14 d of recovery before the next procedure, except for the Belgrade rats, which were euthanized on the fifth day after the second procedure. Each animal underwent 3 laparotomies with biopsy collection, and all of the animals were euthanized at the time of the third collection. None of the animals were treated with antibiotics at any time during this study.

Figure 1. A 2-mm biopsy punch and a package of the absorbable gelatin sponge product.

All of the procedures performed on the animals were conducted in accordance with all applicable federal and institutional regulatory policies. The procedures performed in this study were approved by the IACUC of the University at Buffalo. This study was approved as a pilot study, which, in turn, is the reason for the limited number of subjects used for the experimental procedures.

Phase 1. This phase of the study, the developmental phase, involved 2 Sprague–Dawley rats. The abdominal skin and musculature were incised along the linea alba just caudal to the xiphoid process with a no. 10 scalpel blade. The liver was exposed (a specific lobe was not targeted), and a single sample was obtained using a 3-mm disposable biopsy punch. The defect in the liver created by the biopsy punch was filled with a small quantity of absorbable gelatin compressed sponge (Gelfoam, Pfizer Pharmacia and Upjohn, Kalamazoo, MI) to achieve hemostasis (Figure 1). The abdominal wall was closed with 3-0 absorbable suture (polyglycolic acid suture, Ethicon, Johnson and Johnson, New Brunswick, NJ) in a simple interrupted pattern, and the skin was closed using the same suture in a subcuticular pattern placed in the subcutaneous layer. Absorbable suture was chosen because the suture was buried beneath the skin, that is, no external exposure, and because complete degradation of the suture would not take place within the time intervals between laparotomies. The surgery was repeated in the same manner twice more, with 2 wk of recovery between surgeries. At each successive surgery, the suture material from the previous surgery was removed, and a different area of the liver was biopsied. Previous biopsy sites were identifiable by their white scarring.

Phase 2. This phase was the implementation phase, in which the procedure described for phase 1 was performed on 9 Belgrade rats. During the interval between the second and third liver biopsies, the researcher administered nifedipine (dissolved in DMSO) at a dose of 5 µg/g IP every 24 h for 4 d to the rats in his experimental groups. The Belgrade rats in the control groups received injections of DMSO only. This medication was administered in an effort to replicate results published in a previous study; these published results directly contradicted prior research of the investigator for whom the current technique was developed.[7] The liver biopsy specimens were preserved in 4% paraformaldehyde, sectioned, stained with Prussian blue stain, and visualized by using a 20×-objective and ImageScope Spectrum software (Aperio Vista, CA) to obtain images.

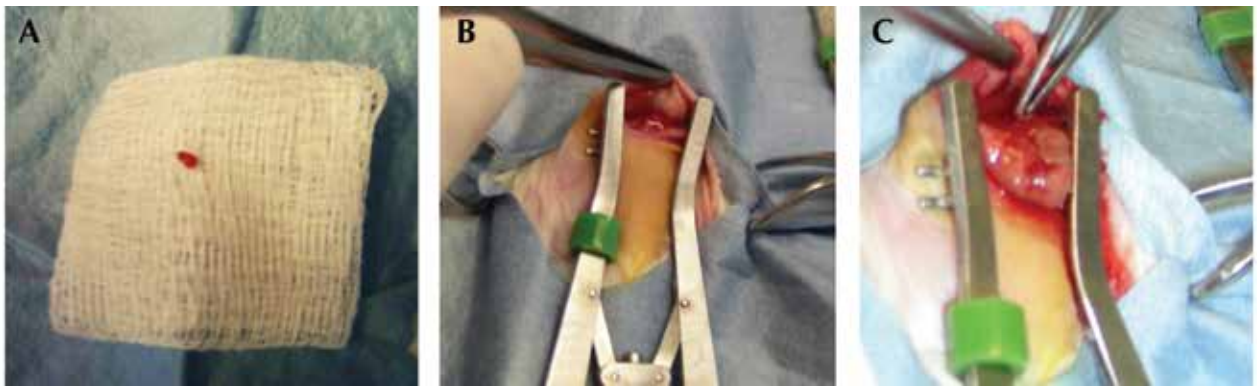

Figure 2. (A) Mouse liver biopsy specimen obtained by using a 2-mm disposable biopsy punch. (B) Exposure of the liver to collect the biopsy. (C) Retrieving the biopsy specimen by using thumb forceps. The circular edge of the biopsy specimen is visible just below the tips of the forceps.

Phase 3. In phase 3, the procedure outlined for phases 1 and 2 was performed on 6 Swiss Webster mice, with minor alterations. The linea alba was incised by using a no. 15 scalpel blade instead of a no. 10 blade. The liver biopsies (one per subject) were obtained in the same manner using a 2-mm biopsy punch (Figure 2) rather than a 3-mm device. In 2 of the mice (age 25 d and 111 d at the time of the first liver biopsy procedure), the defects made in the liver by the biopsy punches were not filled with the gelatin sponge product, and hemostasis was achieved by compression with a sterile cotton swab. The abdominal wall and skin of 4 of the mice were closed with 4-0 polyglycolic acid suture in the same manner as described previously (Figure 3). In the 2 remaining mice (age 25 d and 111d at the time of the first liver biopsy procedure; the same animals as those whose hemostasis was achieved by compression), the abdominal wall was closed with 4-0 polyglycolic acid suture in a simple continuous pattern, and the skin was closed by using suture glue (VetBond, 3M, St Paul, MN; Figure 3).

Statistical analysis. The variables considered to be the most important in this study were the age of the subjects at the time of the first collection, the percentage of weight loss or gain between procedures, and the health status of the subject at the time of the first surgery, which may have been directly related to the strain of the animal. Because this pilot study involved multiple, survival surgeries and because documenting body weight is a simple way to assess the wellbeing of rodents, our statistical analysis attempted to correlate weight loss or gain with the survival of the subjects. The data were processed by a statistician using TI-84 Plus software (Texas Instruments, Dallas, TX). The Pearson χ^2 test was used to evaluate our null hypothesis, which stated that the survival of our subjects was independent of their weight loss or gain throughout the study. A P value of less than 0.05 represented a correlation between the weight loss or gain throughout the study and the survival of our subjects.

Results

Two rats passed away during the study. Both were homozygous recessive (b/b) Belgrade rats. One rat (male) died due to self-inflicted dehiscence from postoperative chewing of the incision, with secondary evisceration after the second liver biopsy surgery; this could occur postoperatively in any strain of rat. The other rat (female) was found dead in her cage on the afternoon after the first biopsy procedure; this rat was pale at the time of surgery and was the oldest rat (563d) in the study. Her death was attributed to the iron-deficiency anemia associated with this strain of rat.

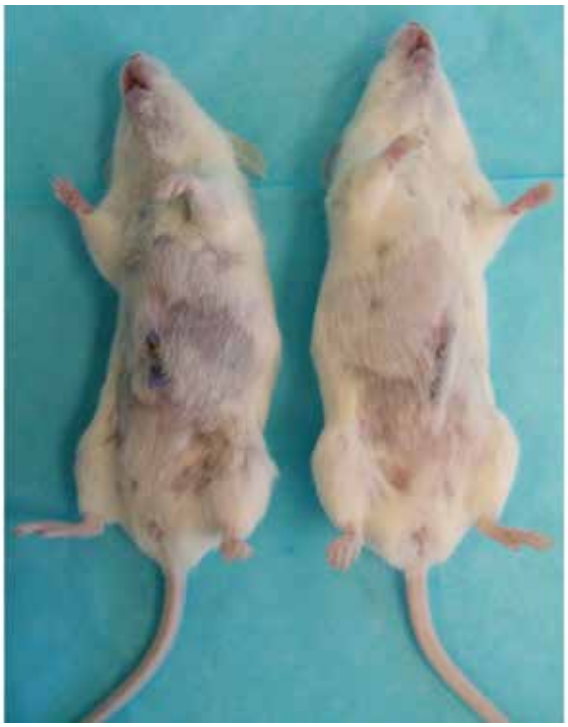

Figure 3. Gross appearance of the mice at the time of the final collection, 6 wk after the initial procedure. The mouse on the left underwent standard closure (suture material was used to close both the abdominal wall and the skin). The mouse on the right underwent the alternative closure (apposition of the abdominal wall with suture and closure of the skin by using suture glue).

During the 2-wk period between the first and second procedures, the Belgrade rats were fed a carbonyl iron diet, which resulted in the development of diarrhea, secondary dehydration, and weight loss (Table 1). The dehydration was treated with daily subcutaneous injections of saline (10 mL), and the diarrhea was treated with daily administration of Pro-Pectalin tablets (1/4 tablet PO every 24 h; Vet Solutions, Fort Worth, TX). The diarrhea resolved after the second procedure, when the carbonyl iron diet was discontinued. Despite the weight loss, all of the rats recovered well from the second procedure, with no additional loss of weight between the second and third biopsy collection procedures.

The 11 rats and 6 mice used for the development of the techniques experienced minimal complications associated with these procedures. One of the younger Swiss Webster mice (25 d

Table 1. Body weight and age of the rats and mice used in the current study

	Weight (g)		Weight gain or loss (%)	Age (d) at first surgery
	Start	End		
Sprague–Dawley rats				
1	500	500	0	247
2	520	510	−2	199
Belgrade rats				
1	428	338	−21	428
2	250	210	−16	428
3	262	218	−17	428
4	244	200	−18	562
5	452	392	−13	486
6	234	198	−15	562
7	238			562
8	280	194	−31	486
9	318	292	−8	563
Swiss Webster mice				
1	40	40	0	38
2	38	38	0	63
3	34	32	−6	111
4	24	28	+17	111
5	10	18	+80	25
6	10	18	+80	25

and 10 g at the time of the first surgery) developed adhesions between the liver and the abdominal body wall which were noticed at the time of the second surgery (2 wk after the first surgery) and when the animal was euthanized (4 wk after the first surgery). A small abscess associated with the cranial aspect of the abdominal incision in this mouse was also noted at the time of euthanasia. This mouse's abdominal musculature was closed with 4-0 polyglycolic acid suture in a simple continuous pattern, and the skin was closed with suture glue for both liver biopsy surgeries. Despite these secondary issues, this mouse continued to thrive and gain weight throughout the study (Table 1). None of the other rodents in the study developed adhesions or abscesses.

Using these methods of collection and closure, both of the principal investigators for whom these procedures were developed were able to obtain sufficient tissue to perform the necessary tests associated with their studies. The investigators did not observe any gross abnormalities at the time of the subsequent liver biopsy collections, and the histology of the current biopsies (Figure 4) was equal in quality to samples collected previously by other means.

The main objectives of this study were to assess the 'survivability'of the subjects in association with a series of laparotomy-assisted liver biopsy collections at 2-wk intervals and to evaluate the quality of both the biopsy material and remaining in situ liver tissue. The sex of the subject, the method of hemostasis after biopsy collection, and the suture materials used were not tested as factors in the recovery or survival of the subjects postoperatively. The survival of all mice and rats that underwent these multiple, survival surgeries was not associated with the weight loss or gain, which we used as our primary index of animal wellbeing. The dramatic weight loss of the Belgrade rats was directly due to the diarrhea and dehydration induced by feeding the carbonyl iron diet. None of the other subjects were fed this diet and none of the other subjects developed diarrhea or dehydration during this study. Referencing our statistical analysis, our χ^2 value was 0.64, and our df value was 2. At the completion of the analysis, our P value was 0.72615, which resulted in the acceptance of our null hypothesis – the survival of the test subjects was independent of the weight loss or gain.

Discussion

The original protocol involved collecting liver biopsies from Belgrade rats (n = 9; 3 wild-type, 3 heterozygotes, and 3 homozygotes) of various ages and of both sexes after they had undergone iron loading for 14 d and then again after treatment with nifedipine, an agent that alters DMT1 activity so that greater quantities of iron can be transported.[6,7] The researcher wanted to perform this pilot project to confirm the results of a previously published study.[6,7] The procedure the researcher proposed involved using percutaneous needle aspirates, and if these did not provide sufficient samples for analysis, they were to excise small sections of a liver lobe by using "a heated, sterile, Teflon-coated scissor to cut and cauterize the lobe."[1] Fine-needle aspirates only provide cell smears for cytomorphologic analysis, commonly used to identify steatosis or dysplastic or carcinoma cells, sometimes requiring additional tissue sampling and histologic examination to confirm the diagnosis.[2] Percutaneous needle aspirates take little time to perform and require minimal handling of the animals, but this method is a blind technique, and needle insertions should follow different pathways when multiple samples are collected at short intervals.[2] After reviewing the protocol, we felt better samples would be obtained if the researcher used disposable 3-mm biopsy punches to collect the liver samples. Furthermore, the *b*/*b* Belgrade rats were anemic and ultrasonography would not be used to guide the collection, increasing the chances that percutaneous aspiration might result in hepatic hemorrhage. In addition, cauterized edges of the liver would require more extensive healing than would the 'clean' sample site created by a biopsy punch.

Liver biopsies are still the most accurate approach to assessing hepatic histopathology, especially in the presence of chronic disease.[9] Liver biopsies can aid in assessing the progression of liver disease, establishing a diagnosis when there is no clear etiology, making decisions regarding treatment choice, and determining whether a treatment was successful.[9] Established liver biopsy techniques include—but are not limited to—wedge biopsy via laparotomy, resection of a liver lobe, and fine-needle aspiration.[1] Of these techniques, fine-needle aspiration is the least invasive. When considering a noninvasive method, the following parameters should be kept in mind: the method should be highly sensitive and specific, technically easy to measure, inexpensive with minimal to no side effects, and useful for monitoring the progression or regression of liver disease both before and after treatment without complication.[9] Fine-needle aspiration might have been considered for the current study if ultrasound guidance could have been used. However, without ultrasound guidance, sampling novel areas at each liver tissue collection time point could not be guaranteed nor could the detection of hemorrhage after aspiration.[2] Since some of the rats were anemic, the risk was significant.

The published data on multiple liver biopsy collection in rodents or collection of liver tissue via survival surgery rather than post-euthanasia are sparse. The earliest described liver biopsy techniques involved excision of entire lobes (partial hepatectomy), requiring ligation of major blood vessels and frequently resulted in substantial blood loss.[14] Although

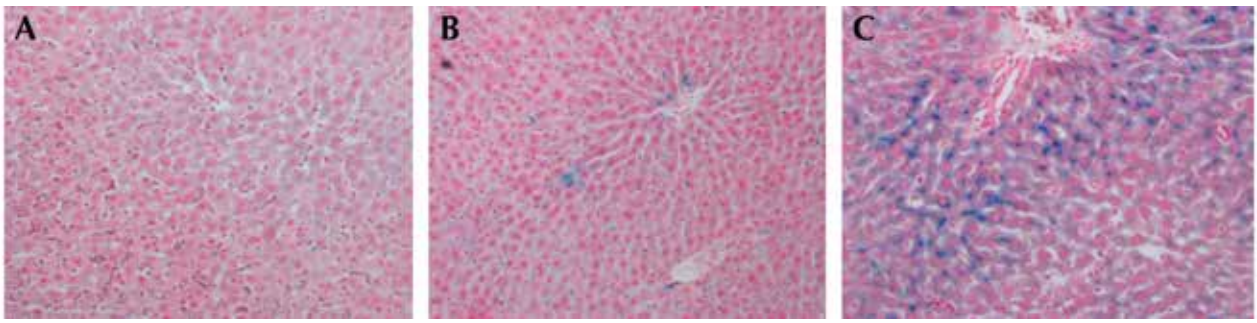

Figure 4. (A) Histology of the liver from a heterozygous Belgrade rat (+/*b*) at the time of the first liver biopsy (10 Nov 09). (B) Histology of the liver from a wild-type (+/+) Belgrade rat after being fed a carbonyl iron diet for 2 wk and at 14 d after the first biopsy (24 Nov 09). (C) Histology of the liver from a homozygous recessive (*b*/*b*) Belgrade rat collected 5 d after the second biopsy procedure (29 Nov 09). The tissues were fixed in 4% paraformaldehyde and stained with Prussian blue stain. Magnification, 20x.

partial hepatectomy provides a large quantity of liver tissue for analysis, it leaves less tissue for subsequent sampling, requires a prolonged postoperative recovery due to blood loss and the need for hepatic regeneration, and has the potential to cause vascular compromise of the remaining liver lobes.

Another technique described in the literature for obtaining multiple liver biopsies in rats used the same approach as our study—abdominal midline incision—and entailed the removal of a section of a lobe.[1] Instead of ligating the blood vessels vascularizing the liver lobe to achieve hemostasis, as in the previously mentioned procedure,bleeding from the stump was controlled by using electrocautery.[1,14] This process of achieving hemostasis induced considerable damage to the remaining tissue of the lobe, thus necessitating the collection of tissue from a different lobe at the next time point.[1] In addition, the technique induced the formation of numerous adhesions between the biopsy sites and the peritoneum and omentum and resulted in scarring at all of the biopsy sites.[1] The scarring and adhesion formation made it difficult to locate and exteriorize liver lobes during subsequent collection surgeries.[1] In comparison, the technique we outline in the current study—despite being invasive due to the creation of an abdominal midline incision—induced minimal adhesion formation and permitted repeated collection from the same liver lobe at subsequent time points.

During the literature search for this study, we discovered one paper that described repeated liver biopsies in mice by using laparoscopy.[11] The procedure used a 1-mm incision to permit the entry of the laparoscope, insufflation of the abdomen to achieve visualization of the abdominal organs, and collection of biopsies by using a 3-French flexible biopsy forceps. Multiple liver biopsies were taken, and the other abdominal organs were examined as well. This novel technique likely would be successful if used to collect serial liver biopsies but requires the use of highly specialized equipment that is not available at every institution. The method we developed during the current study may be more invasive than the previously published procedure, but can be performed by any appropriately trained researcher at any facility.[11]

Another mouse study outlined the use of cryobiopsy for collecting liver samples.[3] The tips of a set of forceps were cooled with liquid nitrogen and then placed on a section of liver; the resulting frozen section of liver was immediately pinched off and removed with the forceps.[3] This approach created extensive damage to the collected samples (ice crystal formation and collapse of sinusoidal cavities).[3] In contrast, the technique developed in the current study does not create such artifacts in the tissue samples and completely avoids the potential for damage to other tissues by the cooled tips of the forceps.

Our focus in our study was to first develop a technique to perform multiple liver biopsies in rats and then to tailor the technique for mice. After considering all of the options (Figure 5), open laparotomy, with the collection of liver samples by using a disposable biopsy punch, seemed to be the best option. This approach was invasive but provided sufficient tissue to perform all of the necessary tests and was associated with only minimal postoperative complications. We used this approach to successfully collect liver biopsies in rats and mice of various ages and strains and in both sexes. The technique is simple to perform, despite the need to create an abdominal incision, and may overall be the least invasive of the invasive methods used to collect repeated liver biopsies. The supplies are easy to acquire, and specialized equipment is not necessary. We have outlined how this technique can be incorporated into any study and how it can provide sufficient tissue to perform many or all of the tests a researcher might need.

The quality of the liver tissue collected by using the method described in the current study is shown in Figure 4. The architecture of the liver is preserved in each of the samples despite the weight loss resulting from the diet-induced gastroenteritis and treatment with nifedipine. The hepatic chords surrounding the triads showed some minor attenuation in the samples acquired at the second and third collections, but the integrity of the hepatocytes was not compromised when compared with those of the specimen obtained at the first collection (Figure 4). Although the use of multiple, survival surgeries is not ideal, the appearance of the hepatic histology coupled with the fact only 2 animals did not survive to the third and final collection support the use of the current proposed method of tissue collection.

Some of the Belgrade rats sustained dramatic weight losses, but these were directly related to the carbonyl iron diet and not to the surgical procedures. This claim is supported by the facts that the weights of the Belgrade rats stabilized after the carbonyl diet was discontinued, the weights of the subjects in the other groups did not change significantly between the first and second procedures, and none of the other subjects developed diarrhea at any time during this study. Figure 3 shows the normal body condition of the younger subset of mice at the time of euthanasia; the study procedures did not prevent weight gain in this younger subset (Table 1).

The present study supports the use of a disposable biopsy punch for the collection of serial biopsies through a laparotomic approach in mice and rats. The method preserves the integrity of the collected samples and that of the tissue remaining within the animal. Caution should be exercised when the subjects are geriatric or anemic, given that the oldest and most anemic animal in our study did not survive. Standard closure of the

Procedure for collecting liver biopsies	Advantages	Disadvantages
Percutaneous needle aspirate	• Not time-consuming • Requires minimal handling • Minimally invasive • No need for anesthesia	• Useful for cellular smears only • May require further sampling • Blind technique—may cause hemorrhage and collection from previously sampled sites
Laparotomy and hepatectomy	• Useful for assessing hepatic histology • Can directly identify sources of bleeding, permitting the placement of effective ligation	• May cause substantial blood loss • Less tissue to sample during subsequent collections • Invasive • Requires anesthesia
Laparotomy using electrocautery to achieve hemostasis	• Useful for assessing hepatic histology • Hemostasis may be achieved quickly	• Damages remaining tissue • Induces scarring and adhesions • Requires special equipment • Invasive • Requires anesthesia
Laparotomy with cryobiopsy	• Minimal bleeding • No specialized equipment	• Extensive damage to collected and remaining tissues • Liquid nitrogen requires special handling • Invasive • Requires anesthesia
Laparotomy using a biopsy punch	• Equipment is easily acquired • Minimal bleeding • Can sample novel areas • Minimal scarring, adhesion formation, and trauma to the surrounding tissues	• May not be optimal for geriatric or anemic animals • Invasive • Requires anesthesia
Laparoscopy with 3-French flexible biopsy forceps	• Decreased invasiveness • Small incision • Multiple samples can be obtained from different sites • Tissue integrity can be assessed	• Requires highly specialized equipment • Requires an experienced and trained operator • Requires anesthesia

Figure 5. Techniques outlined in the Discussion section.

abdominal body wall and skin is preferable to surgical glue, which was associated with adhesions and an abscess in one of the Swiss Webster mice. This approach allows researchers to assess disease processes or treatment responses over time in the same subject, thereby reducing the number of animals needed for such studies.

Acknowledgments

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