— Note —

Experiences with the Identification of Small Rodents

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Own experiences with the identification of mice and rats using ear tags, ear punching, toe clipping (for newborns only) and ear tattooing are given and compared with methods presented in literature. By far, ear tattooing by the newly developed pincers is the most suitable method for marking adult rodents for life. Tattooing is easy to apply, easy to read and not harmful to the animals. For newborn pups on day 4 p. p., the s. c. injection of India ink into the palm of paw is recommended.

The necessity of the identification of laboratory animals has been early recognized and various methods have been recommended. Walker (1935) and Carmichael (1938) both used a system of notches, holes and slits in the ears and toes and tail clipping to identify rats in large colonies. Beach (1938) found that ear tattooing is one extremely simple and practical method of marking rats. He used an apparatus to tattoo designs in human skin. Young rats had been marked for life at weaning. Freeze marking producing white-haired symbols has also been recommended (Farrell and Johnston, 1973).

The proposed guidelines for Good Laboratory Practice (GLP) issued in 1976 by the FDA and in 1981 by the Japanese MHW require unique permanently attached identification number for all exp. animals. Therefore, the usefulness of the various identification methods was recently reconsidered (Avery and Spyker, 1977; Greenham, 1978; Kraus, 1980; Riley and Gwilliam, 1981; Guilloud et al,

1984). The method ultimately chosen should be simple, easy to apply, easy to read and not harmful to the animal (New, 1978). This paper compares the experiences made with 4 different methods, such as ear tags, ear punching, toe clipping and ear tattooing, for the identification of small rodents in our testing facilities. Our aim was not only to draw attention to the new ear tattooing pincers now available but also to give a review of the various methods used and to promote the protection of our experimental animals.

Materials and methods: 520 JCL-ICR-mice purchased from Clea Japan Inc., 500 CRJ-BDF₁ mice purchased from Charles River Japan (all mice identified by ear tattooing), and 10826 SD-JCL-rats bred in our laboratories (5508 identified by ear punching, 5318 by tattooing), all from 5 through 12 weeks old, have been identified in various toxicity studies. A total of 96 SD-JCL rat pups at 4 days old and from 12 litters was also employed.

Ear tags: Ear tags (KN-295 alloy ear tag

for rats and guinea pigs to be attached by KN-296 pincers) have been purchased from Natsume Co., Ltd., Tokyo.

Ear punching: Adult rats have been individually marked according to a code based on the method of Heine (1962) using a chicken toe-punch obtained from poultry-equipment suppliers. A ternary scale with the figures of 0, 1 and 2 has been used. These figures are shown in the form of punching holes, namely no punch=0, a round hole=1, and a marginal punch=2. The test group is indicated by two marks in left ear, whereas each individual number consists of three marks in right ear. By that, 9 (3^2) dose groups and 26 (3^3-1) individual rats can be identified per group excluding No. 000.

Dose group punch=number	Individual rat punch=number, ♂ / ♀
00 =0 (control)	001= 01/51
01 =1	002= 02/52
02 =2	010= 03/53
10 =3	011= 04/54
a. s. o. until	a. s. o. until
22 =8	222= 26/76

for example: male rat No. 105 (female No. 155)
left ear 01=group 1 right ear 012=rat
No. 05 (55)

Toe clipping technique: On day 4 after delivery, litters of rodents are reduced to eight pups. These eight pups are identified by cutting off the first joint of forefoot toes. From 2nd to 5th toe of right paw are pup No. 1-4, and those of left paw pup No. 5-8.

Ear tattooing: The pinnae are stretched using two pincettes by an auxiliary person.

Thereafter, numbers are pressed with tattoo pincers for mice and rats developed by the fine mechanical workshop of Boehringer Ingelheim KG, FRG using nut tongs "KNIPEX" (Rheinwerkzeug, Mainz, FRG) with exchangeable needles from inside the ear, group number on right ear, individual number on left one (it is important to put the needle numbers inversely into the pincers). Thereafter, Hauptner special tattooing ink (H. Hauptner, Solingen, FRG), diluted with a small quantity of distilled water, is firmly painted at the site of imprinting using a brush (Figs. 1 and 2). The success of tattooing depends on the deep penetration of the ink into the puncture channels. The ear's skin at the site of tattooing will gradually clean itself from surplus of ink within 1-week. Then, the identification number is clearly readable.

Very recently, automatic tattoo pincers TZ 2 R and L (E. Becker & Co., Co., Ltd., Castrop-Rauxel, FRG) have been tested. On revolving magazines at the head of the pincers are needle figures easily adjustable (Fig. 3). Otherwise the use is as described above.

Tattooing of 4-days old rat pups: Four methods have been applied using India ink: 1. s. c. injection at back of paw, 2. s. c. injection into palm of paw (canulla inserted in distal direction), 3. needle application and 4. s. c. injection below caudal skin. For the s. c. injection, a 2.5 ml TERUMO^(R) syringe with TOP^(R) needle 21G × 1½"S. B. (0.8 × 38 mm) has been used. In reproduction toxicology studies, after reduction of litter size to eight pups, four males and four females have to be identified on one paw each.

Results: 1. All ear tags caused more or less distinct local lesions, bleeding, swelling and slitting of the ear. The tags were lost in quite a number of cases at some time following application. Exact data cannot be given because the method has been abandoned already in 1972.

2. The ear punching can be considered to be a useful method for the permanently attached identification of rats and was found to be readable. The method was not useful for mice, and not for rats housed in groups be-

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Fig. 1. The equipment needed for ear-tattooing: nut tongs "KNIPEX," two pincettes, Hauptner special tattooing ink and a brush for painting.

Fig. 2. A set of the exchangeable needles of the "KNIPEX" numbered from 0 through 9.

cause of cannibalism. Other disadvantages were the mutilation of the ears, and the necessity to use a key list for the time-consuming deciphering of the marks. Altogether two chronic nodular chondrodermatitis of the ear occurred.

3. The toe clipping technique provides a quick, easy readable and permanent identification method which does not harm the pups more than absolutely unavoidable.

4. 1. The modified "KNIPEX" ear tattoo pincers for mice and rats proved to be extre-

mely useful for the permanent identification of both species (Fig. 4). The tattoo numbers were easy to read also after 2 years at term of carcinogenicity studies. No lesions related to the method were observed. The results with the automatic tattoo pincers TZ 2 in rats were even better because the method is less time consuming, especially for tattooing the individual animal number (left ear), and the figures are more distinctly readable due to the increased number of needles per figure (Fig. 5). A disadvantage is the relatively high

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Fig. 3. The automatic tattoo pincers TZ 2L from E. Becker & Co. Ltd. with revolving needle magazine.

Fig. 4. The ears of a male mouse (No. 2043) killed at term of a 2-year carcinogenicity study. "KNIPEX" tattoo pincers were used for identification at 6-weeks old.

price of the TZ 2, altogether 4 pincers of which have to be purchased one each for right and left ear of both species according to the producer. However, for group identification, the modified "KNIPEX" ear pincers are as useful.

4. 2. Among the tattooing methods used on 4-days old pups, only the s. c. injection into palm of paw resulted in readable marks 3-5 weeks after identification and then, could be replaced by ear tattooing.

Discussion: As in the farm animals, particularly in cattle, also in small rodents the identification by ear tags and buttons succeeded gradually as the technique routinely used until today.

Small numbered plastic or metal tags are commercially available. However, all rats identified with tags show inflammation and bleeding around the ear tag during the first days following application. The inflammation has been present in 30% of these animals at 24-days after application (Guilloud et al., 1984). Ac-

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Fig. 5. A living male rat (No. 40) identified by the automatic tattoo pincers TZ 2L.

cording to the same authors, 13 of 40 rats originally employed have lost their ear tags during an observation period of 23-months. Although no exact data are available, we made the same experiences in our testing facilities and abandoned the ear tags in the early seventies.

Because of these disadvantages, in 1977 we have chosen the identification of rats by ear punching according to Heine (1962). Still very recently, Riley and Gwillian (1981) developed a new ear punch which cuts leaving a clean hole. The method cannot be applied to animals, such as the rabbit, having large marginal ear veins (Short and Woodnot, 1963). The method is useful but time-consuming and it needs care to punch each individual rat according to the key. Thus, technical error is also a problem. Further disadvantages of the ear-punching method are the loss of tissue and the sometimes difficult reading especially of marginal punchings. Fighting among animals can mutilate the ears beyond recognizable readings particularly if housed in groups.

There have been also a very few cases of an edema of the subcutis followed by necrosis of the auricular cartilage. The phenomenon might be related to an anaphylactic reaction after the trauma of ear punching. Following the introduction of this identification method, it was repeatedly seen but never thereafter in

rats tattooed.

It can be concluded that ear punching is a useful method for the identification of singly housed rats. The method is, however, not useful for mice and for grouped rats, and also cannot be recommended in view of the protection of experimental animals.

In 1981, the ear tattooing of small rodents was introduced in our testing facilities. Tattooing is widely used in guinea pigs, rabbits and larger animals. We used Hauptner tattooing forceps for rabbits since 1971. It is the most successful means of identifying these animals (New, 1978).

Until very recently, its application was limited however, because the numbering system used was too large for ears of rodents. Using the newly developed small forceps and needles for ear tattooing of small rodents the method is independent of the number of animal and the duration of the experiment, and has been used successfully in acute toxicity testing as well as in carcinogenicity studies. Tattooing is not only common in man but also contributes to the protection of our experimental animals. Pigmentation of the ear is no limitation because the number is readable from the inner side of the pinnae.

Tattooing is also recommended for newborn rodents over the toe clipping technique (Kraus, 1980). Various methods have been described

in literature: tattooing the palm of the forefoot, the sole of the rear foot, the entire dorsum of the paw or below caudal skin (Geller and Geller, 1966; Schoenborne et al., 1977; Avery and Spyker, 1977; Greenham, 1978; Guilloud et al., 1984). Based on our own experiences, however, among those methods only the s. c. injection of India ink into the palm of paws resulted in an identification readable after weaning and suitable to replace the toe clipping technique which is not only harmful to the animals but also disturbs the investigation of the skeletal system and may influence behavior studies. Also, toe clipping cannot be recommended from the viewpoint of the protection of our experimental animals.

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小動物の個体識別法について

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当研究所では、これまでマウス および ラット等の齧歯類に対する 個体標識法として 耳標識札, 耳パンチ, 前肢の指先切除 (新生仔のみ), 耳入墨法を行ってきたが, 今回我々の得た経験と, 文献等で示された方法との比較を行なった。これらの方法のうち 成体に対しては, 新規に 考案された 入墨器を用いた入墨法が, そ

の動物の生涯にわたって, 入墨された 数字の判読が可能である ことから, 最も適切な 方法と思われる。入墨法は, その方法が 容易で 読み易くしかも 動物に対して悪影響をおよぼさない。新生仔の識別については, 生後 4 日令に四肢の足掌皮下への入墨が推奨される。