alkaloids was other than nicotine. For the tobacco of their time, and for most commercial tobaccos of to-day, this percentage can probably be accepted as typical.

In recent years, however, tobacco breeders have been cultivating low-nicotine strains in an effort to produce milder smoking tobacco. From one such experimental lot of Maryland tobacco there has been isolated, in amount equal to 95 per cent. of the total alkaloids, a base identified as nornicotine. This base had previously been found in tobacco as a minor alkaloid constituent, and it had also been found in other species of *Nicotiana* as the main alkaloid. For example, C. R. Smith[2] found that it constituted about 95 per cent. of the alkaloids of *N. sylvestris*. The occurrence of nornicotine as the predominating alkaloid of *N. tabacum* appears to be a hitherto unrecognized fact. Whether there is an association between low-nicotine strains and nornicotine can not be definitely asserted, but there is some reason for believing that nature compensates for the repression of nicotine by synthesizing the closely related parent alkaloid.

Not only structurally, but also pharmacologically, the two are similar. As a contact insecticide against the bean aphid (*Aphis rumicis* L.), dl-nornicotine was more toxic than dl-nicotine, and about as toxic as l-nicotine.[3] The naturally occurring forms are laevorotatory in both cases, but no insecticide tests with l-nornicotine are known, probably because of its scarcity. Against animals the action of nornicotine was weaker, in one case being only one tenth that of nicotine.[4] From the smoker's standpoint this is fortunate.

The possibilities of nornicotine as a stomach poison against insects have not been explored, chiefly because of its unavailability. Now that the existence of a strain of tobacco containing it has been discovered, it is hoped to prepare a supply of this alkaloid for such tests.

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TATOO PUNCH FOR NUMBERING RATS

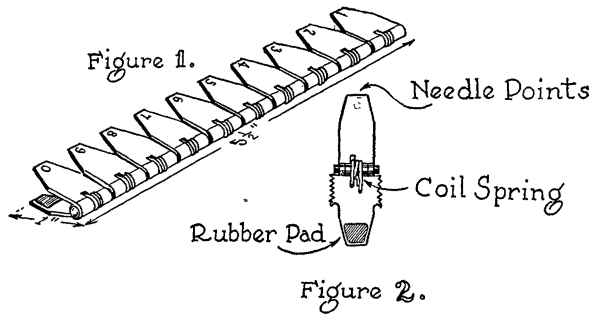
PERSONS handling large experimental colonies of pedigreed albino rats usually invent some personal system of hole-punching or edge-snipping of their rats' ears, which symbols plus sex and cage number may be referred to a ledger to identify the specimens.

But tame albino rats usually face out and become both quiet and inquisitive as one approaches their cage. Making use of this inborn trait, a great deal of time and effort can be saved if one is able to read the individual ledger number of each rat directly in his ears at a glance without even opening the cage door.

In order to be able to do just this, some technicians spend much time in laboriously tatooing the ears of all their rats by means of a needle and India ink. At least 15 minutes is spent on each animal at the time of weaning. This procedure solves the problem admirably, but it is too wasteful of time.

We report here the construction of a handy tatooing punch for rats, which we have tested and found to be satisfactory. It consists of a base plate of sheet metal, $1'' \times 5\frac{1}{2}''$ in dimensions, serrated with 10 teeth on one side and hinged on the other side.

Ten small number plates ($\frac{1}{2}'' \times 1''$) are hinged to the base plate in parallel, as shown in Fig. 1. The number plates are normally held away from the base plate by means of small coil springs. On the serrations of the base plate (Fig. 2) are thin rubber pads. On the inside of the number plates opposite the rubber pads



FIGS. 1 and 2.

are soldered fine needle points so arranged as to outline numerals: 1, 2, 3, 4, 5, 6, 7, 8, 9, 0.

In tatooing rats' ears, one must be sure that the needles go clear through the pinna, and it is well to brush India ink into the holes on both sides to insure the best results. The rubber pad that receives the needle points insures that they pass completely through the ear.

Thus, to employ the tatoo punch properly the operator takes the punch in his right hand, inks the number desired by means of a stubby watercolor paint brush, grasps in his left hand the rat to be numbered, and with the index finger and thumb of the right hand closes the appropriate numeral plate firmly on the rat's ear

[2] C. R. Smith, *Jour. Econ. Ent.*, 30: 724–727, 1937.

[3] C. H. Richardson, L. C. Craig and T. R. Hansberry, *Jour. Econ. Ent.*, 29: 850–5, 1936.

[4] A. Bergwall, quoted by M. Ehrenstein, *Arch. der Pharm.*, 269: 627–59, 1931.

so that the inked needles pass through the ear and their tips penetrate the rubber pad. By releasing pressure the punch springs open. The operator then dabs the protruding needle points and removes the punch, at which time the needle points carry ink back into the holes as they are being withdrawn. Finally the operator brushes both sides of the ear vigorously in order to work the ink deeply into all the needle holes.

The chief difficulty encountered in preparing such a tatooing device is to have the needles fine enough and numerous enough that when a rat is tatooed at 3 weeks of age, the tatooed points will not separate enough to distort the numeral during subsequent growth of the pinna, because later the numbers increase about ⅓ both in length and breadth.

Likewise the numerals must be small enough that at least two of them may be placed side by side in each ear.

By using two numbers in each ear one may distinguish up to 10,000 individuals and by employing three numbers one may continue the series up to 1,000,000.

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A TESTED METHOD OF GROWING STENTOR COERULEUS

THE heterotrich protozoan *Stentor coeruleus* is valuable for many laboratory purposes but is difficult to maintain in culture. Several attempts were made to grow it according to methods described in the literature (Hetherington[1]; Turner,[2] Brandwein[2]), but none of these produced a satisfactory supply of specimens. Finally, a standard method was developed which has yielded specimens in abundance for more than a year. The method is described in the following paragraph:

Gallon jars are nearly filled with spring water, and to each is added 10 cc of an extract of lettuce, prepared by boiling 10 grams of fresh lettuce leaves in 100 cc of distilled water until about 50 cc has been evaporated. After 12 to 24 hours the water begins to appear cloudy due to bacteria. Then specimens of *Stentor coeruleus* and *Chilomonas paramecium* are put into the jars. Every few days, when the water shows signs of becoming clear, fresh lettuce extract is added. Whenever (usually two to four weeks) the stentors begin to multiply less rapidly, new cultures are started by transferring some stentors and chilomonads from the old cultures to jars of new medium. The jars are kept at room temperature in light of very low intensity or in darkness.

It is not known whether the stentors depend mainly for food upon the chilomonads or upon the bacteria. It is known, however, that the chilomonads are ingested readily. Rotifers which may be present in the cultures seem to cause no trouble, but paramecia must be kept out because they consume the bacteria so quickly that the stentors are deprived of their food supply.

The jars must be kept in light of low intensity or in darkness to prevent the excessive growth of unicellular green algae. Even in moderate light the algae gradually cause the culture medium to become green. If this condition is approached the stentors must be transferred to fresh medium.

No more than 10 cc of lettuce extract may be added to a jar at one time because an excess will cause bacteria and yeast cells to increase so much that the culture becomes too acid. The hydrogen-ion concentration of the cultures must remain between pH 6.2 and pH 7.8. These limits will ordinarily not be exceeded if the bacteria do not become too numerous.

Some difficulty may be experienced from the growth of aquatic fungi. The stentors tend to become entangled in the mycelia of these molds, making it difficult to remove them without injury. By stirring the cultures vigorously every few days, the mycelia are dislodged from the sides of the jars and settle to the bottom, where they cause no further trouble. If no lettuce infusion is added for some time, the stentors accumulate in the mats of mycelia on the bottom of the jars, but if more lettuce infusion is added they again collect near the surface of the water.

If it is desired simply to maintain a culture of stentors with a minimum of effort, fresh lettuce leaves may be put into the culture jars. But if this is done the stentors attach to the leaves. The advantage of using an extract of lettuce is that the stentors attach to the sides of the jars near the surface, whence they can be easily removed with a pipette.

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[1] A. Hetherington, *Archiv. für Protistk.*, 76: 118–129, 1932.

[2] J. P. Turner and P. Brandwein, "Culture Methods for Invertebrates," pp. 60 and 64. Ithaca, N. Y.: Comstock Publishing Company.

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