more probable that the difference is due to a slightly different spectrophotometric technique and the use of different instruments.

Since the high-melting vitamin A alcohol and the crystalline palmitate present possibilities for biological research and the standardization of vitamin A preparations, it is important that their stability under various conditions be known. We have made preliminary storage tests on the palmitate, distilled esters from a fish-liver oil, vitamin A 2-naphthoate (kindly supplied by Dr. T. H. Mead) and beta carotene, the present international standard, and found that they are equally stable in refined cottonseed oil at comparable concentrations when exposed to air in the dark. The crystalline vitamin A palmitate decays more rapidly than the naphthoate on exposure to air. It is hoped to make a further report on the stability of these materials.

A preliminary biological assay of the crystalline vitamin A alcohol has shown that its potency is greater than 2,700,000 U.S.P. units per gram. It is planned to determine the biological potency of both crystalline vitamin A alcohol and crystalline vitamin A palmitate as precisely as possible. The results will be reported separately.

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## THE EFFECT OF SALICYLATE ON THE OXYGEN UPTAKE OF THE TUBERCLE BACILLUS

UNLIKE most other bacteria the tubercle bacillus does not readily oxidize carbohydrates, amino acids, hydroxy acids, etc., when these substances are added to suspensions in the Warburg apparatus. As shown in Fig. 1, the addition of 1.0 mg of sodium salicylate (o-hydroxybenzoate) to the bacteria suspended in 2.0 cc of M/20 phosphate buffer pH 6.7 more than doubles the oxygen uptake. A corresponding increase in $CO_2$ production also occurs. The bovine strain $B_1$ was used. It was grown on beef glycerine infusion broth and the floating masses were removed with a loop and suspended in sterile saline in Hopkins tubes. These were then centrifuged at 2,000 r.p.m. for 15 minutes and the saline replaced by sterile buffer, so that 0.1–0.2 cc of the packed bacteria were suspended in 1.0 cc of buffer. A glass rod the diameter of which was just smaller than the narrow part of the Hopkins tube broke up the cell masses and gave an even suspension. Fig. 1 shows that benzoate also has an effect on the oxygen uptake. On the other hand, p- and m-hydroxybenzoates and methyl salicylate are without action, as is acetylsalicylate until the acetyl group has been hydrolyzed off. p-aminobenzoate has no action and

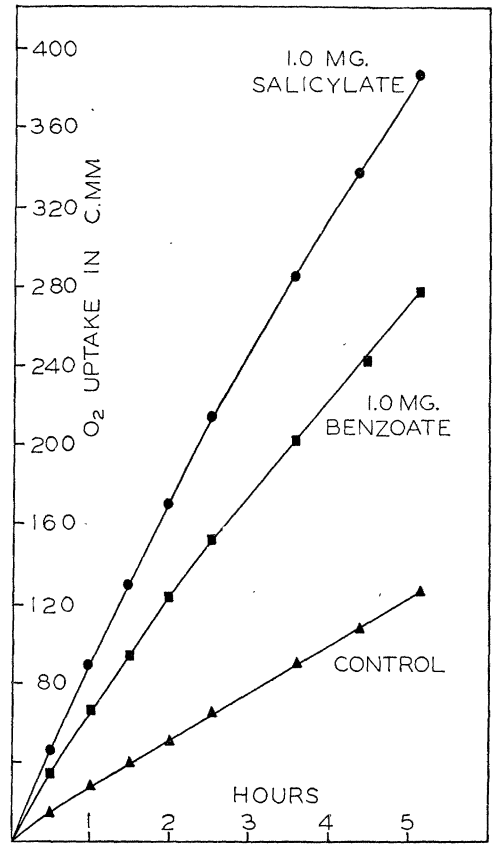


FIG. 1. The oxygen uptake of 0.5 cc of the suspension of tubercle bacilli alone and with salicylate and benzoate at pH 6.7 and 37° C.

o-aminobenzoate only a slight one, but when the latter is added with salicylate it inhibits the salicylate effect. o-aminosulfonic acids have not yet been tried.

When 0.1–0.2 mg of salicylate is used the oxygen uptake is proportional to the concentration, indicating that the salicylate is being oxidized as a substrate. No definite end-points were obtained. The results do not prove that salicylate is a normal metabolite of the tubercle bacillus but suggest that it or compounds of similar configuration may be important. The fact that p-aminobenzoate has been shown to be a metabolite of certain streptococci[1] indicates that substituted benzoates may play a part in bacterial metabolism.

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## NORNICOTINE AS THE PREDOMINATING ALKALOID IN CERTAIN TOBACCOS

NICOTINE has long been considered the main alkaloid of *Nicotiana tabacum*, ordinary tobacco. This view is based upon the finding of Pictet and Rotschy,[1] in 1901, that only 2.5 per cent. of the total tobacco

[1] D. D. Woods, *Brit. Jour. Exp. Path.*, 21: 74, 1940.
[1] Amé Pictet and A. Rotschy, [Paris] *Acad. des Sci. Compt. Rend.*, 132, 971–2, 1901.

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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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## TATOO PUNCH FOR NUMBERING RATS

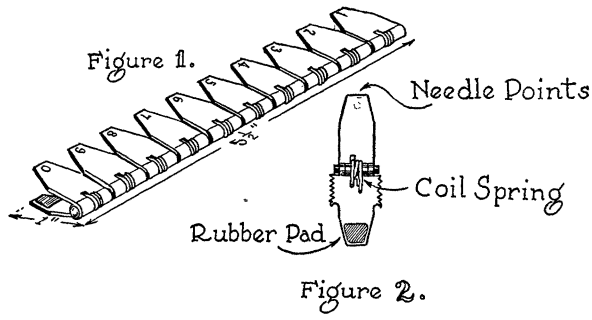
PERSONS handling large experimental colonies of pedigreed albino rats usually invent some personal system of hole-punching or edge-snipping of their rats' ears, which symbols plus sex and cage number may be referred to a ledger to identify the specimens.

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.