cretion rate diminishes with time. This decrease in excretion rate should parallel the decrease in absorption rate of the implant because of area changes which occur during absorption. When such a plot is made from the data presented in Table I of Latven and Welch (9), a decrease in excretion rate with time is observed. However, it cannot be determined from their data the values of the initial and final areas of the implants. Further, it should be possible to equate implant absorption rate to the urinary excretion rate of free and metabolized drug when implant surface area and fraction of the total dose recovered are considered and when absorption is solution rate limited.

An animal implanted with a drug pellet of a geometric form such that only small decreases in area occur with absorption may be likened to one given a continuous infusion of a drug when absorption from implant is solution rate limited. For the infused animal the relationship

$$Ri = Re/f \qquad \text{(Eq. 1)}$$

should apply after equilibrium is established, assuming drug removal processes are first order which is the usual case, where Ri is the constant rate of infusion, Re is the rate of urinary excretion, and f is the fraction of the total dose excreted in the urine in a time infinite in terms of the experiment. A similar relationship should hold in the case of implants after surface area corrections are made. Then the mean absorption rate per mean area, $\bar{R}/\bar{A}$, should equal the mean excretion rate per mean area, $\overline{Re}/\bar{A}$, divided by the fraction, f

$$\bar{R}/\bar{A} = (\overline{Re}/\bar{A})/f \qquad \text{(Eq. 2)}$$

assuming that no deposition of drug occurs at another site (*i.e.*, the kidney) (12, 13).

$$\bar{R}/\bar{A} \text{ per pellet} = \frac{(W_i - W_f)/t_i}{(A_i + A_f)/2} \qquad \text{(Eq. 3)}$$

$$\overline{Re}/\bar{A} \text{ per pellet} = \frac{(W_e/N)/t_e}{(A_i + A_f)/2} \qquad \text{(Eq. 4)}$$

where W_i and W_f are the initial and final mean pellet weights, t_i is the time of implantation, A_i and A_f are the initial and final areas of the implant, W_e is the weight of total drug recovered in the urine in time t_e, N is the number of pellets used, and t_e is the time of excretion.

TABLE III.—MATERIAL BALANCE: SHOWING THAT $\bar{R}/\bar{A} \cong (\overline{Re}/\bar{A})/f$

Rat	$\bar{R}/\bar{A}$ Gm./hr./cm.²	$(\overline{Re}/\bar{A})/f$ Gm./hr./cm.²
A	1.74×10^{-4}[a]	1.66×10^{-4}[b]
B	1.57×10^{-4}	1.47×10^{-4}
C	1.49×10^{-4}	1.40×10^{-4}

[a] $\bar{R}/\bar{A}$ per pellet $= \dfrac{(Wi - Wf)/ti}{(Ai + Af)/2} = \dfrac{(0.0508 - 0.0342)/119.2}{(0.864 + 0.740)/2} = 1.74 \times 10^{-4}$

[b] $(\overline{Re}/\bar{A})/f$ per pellet $= \dfrac{(We/N)/te}{(Ai + Af)/2}/f = \dfrac{(1/4 \times 0.0528)/118.9}{(0.864 + 0.740)/2}/0.837 = 1.66 \times 10^{-4}$

Table III shows the agreement between the experimental values of $\bar{R}/\bar{A}$ and $(\overline{Re}/\bar{A})/f$. Similar agreement was also observed when a different method of calculating $\overline{Re}/\bar{A}$ was used (13). The value of the $(\overline{Re}/\bar{A})/f$ is slightly smaller than that for $\bar{R}/\bar{A}$. This is expected since time in the beginning of the experiment is required for the establishment of equilibrium in drug absorption, distribution, metabolism, or excretion of drug; *i.e.*, t_e by this method is slightly larger than it should be.

REFERENCES

(1) Ballard, B. E., and Nelson, E., *J. Pharmacol. Expt.. Therap.*, 135, 120(1962).
(2) Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 128, 537(1939).
(3) Folley, S. J., *Nature*, 150, 403(1942).
(4) Ballard, B. E., and Nelson, E., *Am. J. Vet. Res.*, 23, 678(1962).
(5) Ballard, B. E., and Nelson, E., *Arch. Int. Pharmacodyn.*, 133, 206(1961).
(6) Eidelsberg, J., Bruger, M., and Lipkin, M., *J. Clin. Endocrinol. Metab.*, 2, 329(1942).
(7) Dorfman, R. I., and Hamilton, J. B., *ibid.*, 1, 352 (1941); Dorfman, R. I., Horwitt, B. N., Shipley, R. A., Fish, W. R., and Abbott, W. E., *Endocrinology*, 41, 470 (1947).
(8) Bennett, H. G., Jr., Biskind, G., and Mark, J., *Am. J. Obstet. Gynecol.*, 39, 504(1940).
(9) Latven, A. R., and Welch, A. D., *J. Pharmacol. Exptl. Therap.*, 91, 161(1947).
(10) Kimeldorf, D. J., *Endocrinology*, 43, 83(1948).
(11) Bromberg, Y. M., Brzezinski, A., and Sulman, F., *Proc. Soc. Exptl. Biol. Med.*, 64, 354(1947).
(12) Nelson, E., *Nature*, 189, 928(1961); THIS JOURNAL, 50, 912(1961).
(13) Ballard, B. E., Doctor of Philosophy Thesis, University of California, Berkeley, 1961.

A Field Method for Alkaloid Screening of Plants

By C. C. J. CULVENOR and J. S. FITZGERALD

An alkaloid screening procedure is described which is suitable for use in the field with fresh plant material and can be carried out in a sufficiently short time to enable a chemist to keep pace with a collecting botanist.

IN VIEW of a strong world-wide interest in the discovery and isolation of new plant alkaloids, the following observations on a simple screen test suitable for field use may be of value. The method was designed for use by a botanist-chemist team collecting in remote and sparsely settled areas not previously explored for alkaloid-bearing plants. In making a test in the field under these circumstances there are great advantages: only positive samples need be collected, thus avoiding drying and despatch of most of the new species encountered; and samples giving strongly positive tests can be collected in bulk immediately, eliminating the need to revisit the area for this purpose. To achieve these advantages, a species should be tested when first encountered rather than collecting and keeping it until camp is set up in the evening. Thus, speed of operation and simplicity of procedure are essential. The

Received May 22, 1962, from the Division of Organic Chemistry, Commonwealth Scientific and Industrial Research Organization, Chemical Research Laboratories, Melbourne, Australia.
Accepted for publication June 11, 1962.

method described allows a plant to be tested in about six minutes, and the full requirements of chemicals and apparatus for one or two days' operations to be housed in a small carrying-case.

EXPERIMENTAL

About 2–4 Gm. of fresh plant material, preferably leaf and soft stems, is ground in a 3 in. unglazed porcelain mortar with a small amount of clean sand and sufficient chloroform to yield a thick slurry. Fine grinding to break down cell structure is important for rapid extraction. Ammoniacal chloroform (10 ml., *N*/20 with respect to ammonia) is added and the mixture stirred for about one minute before filtering the chloroform into a 5 × 1/2 in. test tube. Sufficient recovery of extract is obtained by pressing the material in the filter with a finger. Dilute sulfuric acid (2 *N*, 0.5 ml.) is added, the test tube shaken with a finger closing the end, and the phases allowed to separate (one minute or less is usually sufficient). The aqueous layer, or portion thereof, is removed with a dropper whose tip is fitted with a cotton wool plug for filtering and breaking emulsions. After removing the cotton wool and any chloroform in the dropper, two or three drops of the aqueous solution are placed in two 1 in. × 1/4 in. test tubes for testing with Mayers reagent or silicotungstic acid. The density of precipitate formed is assessed on a + to ++++ basis. Mortar, pestle, and filter funnel are wiped immediately with paper tissue but not washed after a negative test. Test tubes (5 × 1/2 in.) and droppers are emptied and re-used without washing after a negative test. After a positive result, mortar, pestle, and funnel are rinsed with chloroform and re-wiped, and test tube and dropper are rinsed with water from a wash-bottle. These precautions are sufficient to avoid contamination of a sample by the preceding one, while keeping the time required and usage of water at a minimum.

Fig. 1.—Alkaloid testing kit. At rear: Water wash bottle (100 ml.); chloroform containing *N*/20 ammonia (250 ml.); chloroform (250 ml.); 2 *N* sulphuric acid with calibrated dropper (100 ml.); Mayers reagent (50 ml.); silicotungstic acid solution (50 ml.); Center: Droppers with cotton wool filter plug (30); 2 in. funnel; 5 in. × 1/2 in. test tubes (24); 1 in. × 1/4 in. test tubes (several dozen). Front: Folded 11 cm. filter papers (24), 3 in. × 3/4 in. test tube calibrated to measure 10 ml.; 3 1/2 in. mortar; pestle; scissors; sand (250 ml. bottle).

The carrying case is illustrated in Fig. 1. All items are well embedded in balsa wood to prevent breakage or spillage under rough conditions. Polystyrene foam, although lighter in weight, is not satisfactory in place of balsa wood since it collapses wherever chloroform is spilled. Under field conditions, restocking is carried out each evening.

RESULTS

Results of the field test have been compared with those of a laboratory method which employs extraction of 20 Gm. dried milled plant with hot ethanol, removal of the ethanol, partitioning of the residue between ether and aqueous ammonia, and recovery of base from the ether with aqueous acid. The initial comparison was of results obtained in the field on fresh leaf material, with those obtained by the laboratory procedure on the same samples after drying and storing for up to three months. A limitation of the field method is that it misses all quaternary alkaloids, and the following comparisons are based only on the test for tertiary bases. The two methods gave identical results on 114 samples out of 147, 104 being negative, 4 weakly positive, and 6 strongly positive. The field test gave negative results for 14 species which were weakly positive by the laboratory method. The field test gave positive results (7 weak, 12 medium to strong) with 19 species for which the laboratory method gave only doubtful positives.

In view of the different condition of the samples, this comparison is useful only as evidence that the field method is a reliable screening procedure. Since our experience is that a weakly positive result (1+) in the laboratory test indicates that a plant is rarely worth further examination, the negative field result for 14 species in this category would not lead to any useful material being lost. The 12 species giving medium or strong field tests but weak laboratory tests may contain amines of very low molecular weight or suffer decomposition of alkaloid during drying of the plant material.

In order to compare the two methods more closely, a second group of 49 species was examined with both tests made from the same sample of fresh leaf material. Forty-four species gave the same result in both tests, 30 being negative, 10 strongly positive, and 4 weakly positive. Five species were strongly positive in the field test but only weakly positive by the laboratory method.

Our conclusion is that the field method with fresh material is a reliable screening procedure in so far as no species are missed which would have attracted further attention on the basis of the laboratory method. Since the field test is much simpler and can be performed more rapidly than the other, it may find application in circumstances other than that for which it was designed.