stopped when 15–20 per cent of the cystine had disappeared. Whether this is due to product inhibition has not been ascertained, since all the products, particularly the sulphur-containing ones, have not been identified.

We are at present purifying the soluble enzyme and investigating its properties in detail. These results will be reported elsewhere. The present work was supported by a grant from the National Institutes of Health, U.S. Public Health Service.

MOSHE TISHEL
MENDEL MAZELIS

Department of Food Science and Technology,
University of California,
Davis, California.

[1] Cavallini, D., DeMarco, C., Mondovi, B., and Mori, B. G., *Enzymologia*, **22**, 161 (1960).
[2] Flavin, M., *J. Biol. Chem.*, **237**, 768 (1962).
[3] Flavin, M., and Slaughter, C., *J. Biol. Chem.*, **239**, 2212 (1964).
[4] Friedemann, T. E., and Haugen, G. E., *J. Biol. Chem.*, **147**, 415 (1943).
[5] Nakamura, T., and Sato, R., *Biochem. J.*, **86**, 328 (1963).

Purification of Insulin labelled with Iodine-131

THE procedure described by Hunter and Greenwood[1] with some minor modifications has been used in this laboratory for small-scale preparation of iodine-131-labelled insulin. About 1·2 mc. iodine-131 from Kjeller is used in a volume of less than 0·025 ml. for the labelling of 5 μg of purified insulin. The iodination efficiency is usually in the region of 60–80 per cent, giving a specific activity of about 150 μc. iodine-131 per μg insulin.

Various purification techniques for iodine-131-labelled insulin have been reported[2–4]. In some experiments we used the method devised by Banerjee and Gibson and found it possible to purify the reaction mixture in one operation only, thus omitting the dialysing step for removal of free iodine-131.

0·2 ml. of human serum albumin solution (20 mg albumin per ml. buffer) was added to the reaction mixture prepared according to Hunter *et al.* A column of 1 g 'Sephadex *G*–50' gel (fine) was prepared, and 0·07 M sodium barbitone buffer (*p*H 8·6) was used as the elution medium. The column was about 600 mm long and had an inner diameter of 6 mm. After slow elution, the various fractions were examined for radioactivity and analysed by paper chromatography and paper electrophoresis.

The elution pattern obtained from three experiments was as follows: (1) Altered or damaged insulin which came out together with the serum protein and some unaltered insulin in the first 6 ml. eluate showed 5–25 per cent of the total activity. (2) Purified insulin which held 35–57 per cent of the total activity and 2–4 per cent impurities was contained in the next 2 ml. portion. The impurity is assumed to be insulin which has been altered when the material passes through the column. This fraction was collected and diluted in 0·5 per cent serum albumin solution immediately after elution and then subjected to deep freezing. The last portion (8–25 ml.) mainly contained free iodine-131. Usually less than 10 per cent of the total activity is absorbed to the column.

Determination of pure and altered insulin was performed using ascending paper chromatography and two different solvents (barbital buffer, *p*H 8·6 (ref. 4), and a mixture of *n*-butanol, acetic acid and water[5]). Free iodine-131 was determined by paper electrophoresis in which 0·1 N sodium hydroxide was used as electrolyte[6].

ÅGE HØYE

c/o Institutt for Atomenergi,
P.O. Box 40, Kjeller, Norway.

[1] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).
[2] Manipol, V., and Spitzy, H., *Int. J. Appl. Rad. Is.*, **13**, 647 (1962).
[3] Banerjee, R. N., and Gibson, K., *J. Endocrin.*, **25**, 145 (1962).
[4] Merimee, T. J., and Prout, T. E., *Metabolism*, **14**, No. 2, 115 (1965).
[5] Robinson, F. A., and Fehr, K. L. A., *Biochem. J.*, **51**, 298 (1952).
[6] Grassini, G., and Lederer, M., *J. Chrom.*, **2**, 326 (1959).

Isolation of the Scent-trail Pheromone of an Australian Termite

THE trail-laying capabilities of termites have been known for many years and, quite recently, this activity has been shown[1,2] to be based on pheromones produced by the sternal gland. Both primitive and advanced termites possess such a gland, which consists of a rather diffuse structure beneath the fifth abdominal sternite[3]. In two species, namely *Zootermopsis nevadensis* (Hagen) (Hodotermitidae)[1,2] and *Nasutitermes corniger* (Mots.) (Termitidae)[2], extracts of the appropriate region, or indeed of the whole insects, could be used to lay artificial trails that were readily followed by the parent termites. However, no clear indication of the chemical nature of the pheromones involved has yet been published.

In the present work, a scent-trail pheromone common to several Australian species of *Nasutitermes* has been isolated and characterized as an unsaturated diterpenoid hydrocarbon, $C_{20}H_{32}$. The mainstay of the investigation has been the southern, ground-nesting termite *Nasutitermes exitiosus* (Hill). This species has the advantage that it is available in large quantities and it shows a comparatively "docile" disposition in culture which is admirably suited to the requirements of a reliable biological test. In the absence of repellent substances, both soldiers and workers readily follow artificial trails traced on paper from ethereal pheromone, over the range of concentrations about 10^{-8}–10^{-5} g/ml., although above and below these limits there is little or no response. Thus, to test a preparation for trail-laying activity, it is necessary to use several successive tenfold dilutions in order to cover the possibility that pheromone may originally be present at too high a concentration.

In such a test, the *exitiosus* pheromone was shown to be extractable from homogenized termites by light petroleum, and to be stable to alkaline hydrolysis. The unsaponifiable fraction of the extract in light petroleum was refined by means of chromatography on a dry column of alumina (activity I). Under these conditions, inactive saturated hydrocarbons were the first to emerge, followed by colourless unsaturated hydrocarbons and later by bright orange-yellow β-carotene (identified by its characteristic absorption between 300 and 550 mμ). Trail-laying activity was confined to the colourless unsaturated fractions, and vacuum distillation of these, to which a little *n*-eicosane had been added as a carrier, afforded an active, volatile fraction, b.p. 120°–220° (bath temperature), at 25 mm of mercury.

The active fraction was next subjected to thin-layer chromatography on silica gel, with light petroleum as the mobile phase. Treatment of a longitudinal section of the chromatogram with cold concentrated sulphuric acid revealed five major bands as purplish spots, fading to pale brown, with approximate R_F values of 0·0, 0·15, 0·4, 0·6 and 0·85. Saturated materials travelled with the solvent front. Trail-laying activity was associated with the R_F 0·4 zone, and elution of this separated portion of the chromatogram with light petroleum gave a few milligrams of a highly-active, colourless oil. Gas chromatography at 175° (on a Pye 'Argon' instrument, with a 120-cm by 4-mm column, packed with 10 per cent silicone oil on 'Celite', and a gas flow of 75 ml./min) showed that this material was made up of two components with retention times of 2·7 and 14·5 min respectively, and suggested molecular complexities of C_{15} and C_{20}. Resolution of the binary mixture was achieved either by preparative gas chromatography at 225° (120-cm by 1-cm column, packed with 10 per cent 'Apiezon-*L*' on 'Celite', and a gas flow of 100 ml./min), or by short-path, fractional distillation at 25 mm. Progress in either case was monitored on the analytical gas chromatograph. The former method gave products of high purity, but recoveries were low and the latter was preferred for most purposes. Only the higher boiling substance was active in the trail-laying test and

© 1966 Nature Publishing Group