The original spectra reproduced in Fig. 1C (extract No. 1) correspond to a single carbon tetrachloride extraction of an experimental system initially containing 70 mgm. of *n*-heptane. The characteristic bands of hept-1-ene at 909 cm^{-1} and 1,634 cm^{-1} are clearly visible without scale expansion (curve 1) and much more marked with a 10-times scale expansion (curve 2). On curve 2, the absorption band at 3,058 cm^{-1} forms a distinct shoulder on the adjacent band of *n*-heptane. The spectra in Fig. 1D have been recorded on a sample of carbon tetrachloride which had been used for the successive extraction of 9 experimental systems, each containing initially about 40 mgm. *n*-heptane. On these spectra, the three aforementioned bands are clearly visible and also the band at 1,815 cm^{-1} . The absence of the fifth characteristic band (992 cm^{-1}) is due to the fact that the very strong absorption of carbon tetrachloride in this range cannot be exactly compensated for and was actually overcompensated.

The chemical structure of the olefine formed in the reaction is demonstrated by the absence of the single characteristic absorption band³ of *trans*-hept-2-ene and *trans*-hept-3-ene. The absorption bands of the corresponding *cis*-heptenes³ are not interfering with the bands of the other *n*-heptenes and are too weak to be apparent in the carbon tetrachloride mixtures. The complete absence of the characteristic bands of 1-*n*-heptanol, *n*-heptanal, heptanoic acid and methylheptanoate should be also noted.

J. CHOUTEAU

Faculté des Sciences Marseille, 1.

E. AZOULAY

J. C. SENEZ

Centre National de la Recherche Scientifique,
Centre de Recherches
Scientifiques Industrielles et Maritimes,
Marseille, 6.

¹ Azoulay, E., and Senéz, J. C., *C.R. Acad. Sci., Paris*, **30**, 458 (1958).

² Senéz, J. C., and Azoulay, E., *Biochim. Biophys. Acta*, **47**, 307 (1961).

³ Sheppard, N., and Simpson, D. M., *Quarterly Revs.*, **6**, 1 (1952).

Increased Sensitivity of Ethylenediamine Analysis for Norepinephrine

ATTEMPTS have been made to increase the sensitivity of analysis for catecholamines using the ethylenediamine condensation method described by Weil-Malherbe and Bone¹. The objective was to be able to determine catecholamine-levels of 0.05 μgm . and below. Examination of the intensity of fluorescence of norepinephrine after various periods of heating demonstrated, in agreement with others^{2,3}, that 1-2 hr. heating at 50° C. was optimum. It was also possible to obtain the same intensity of fluorescence by permitting the reaction to proceed at room temperature for about 7 hr. or at 5° C. overnight. At 50° C. the intensity of fluorescence was found to be maximum in 20 min. for epinephrine and in 40-50 min. for dopamine. When using the Klett fluorimeter, the fluorescence for epinephrine was stable while that for norepinephrine and dopamine was not.

As reported previously², intense ultra-violet light caused a rapid decrease in intensity of fluorescence. In the Klett fluorimeter, the intensity of the ultra-violet light was great enough to cause a significant drop in fluorescence during the time it took to null the meter. Exposure to the incident beam for more than 5 sec. could be avoided by measuring the deflexion of the galvanometer beam rather than

using the galvanometer as a null point meter. During this time the decrease in fluorescence was minimal. With the Aminco-Bowman spectrophotofluorometer, the fluorescence from norepinephrine and dopamine decreased more slowly, and, for practical purposes, could be considered stable. When the proper activation and fluorescent wave-lengths were used, the intensity of the fluorescence from norepinephrine was somewhat greater than from epinephrine. This improved sensitivity of the method still would not permit the analysis of 0.05 μgm . or less of norepinephrine. Examination of the extractability of the fluorescent product of norepinephrine with various volumes of isobutanol yielded the results given in Table 1. It was observed that a 12-fold concentration of the fluorescent product is possible by decreasing the volume of isobutanol from 3.0 to 0.25 ml. By using micro-cuvettes and the following slit arrangement: (1/16, 1/32, 1/8, none, none, none and 1/16 in. openings for slits No. 1, 2, 3, 4, 5, 6 and 7 respectively), one is able to detect as little as 0.0075 μgm . of norepinephrine.

Table 1. FLUORESCENCE OBTAINED WITH 0.2 μgm . OF NOREPINEPHRINE FOLLOWING EXTRACTIONS OF 5 ML. OF 0.2 N ACETIC ACID WITH VARIOUS VOLUMES OF ISOBUTANOL

Sample No.	Isobutanol Vol. (ml.)	Fluorescent units
1	3.00	0.012
2	1.50	0.024
3	0.75	0.045
4	0.50	0.075
5	0.25	0.162

This simple modification of the procedure has been used successfully for the determination of catecholamine-levels in isolated rabbit arterial strips.

HERBERT SHEPPARD

JOSEPH ZIMMERMAN

Research Department,
CIBA Pharmaceutical Co.,
Summit, New Jersey.

¹ Weil-Malherbe, H., and Bone, A. D., *Biochem. J.*, **51**, 311 (1952).

² Erne, K., and Canbäck, T., *J. Pharm. Pharmacol.*, **7**, 248 (1955).

³ Burn, G. P., and Field, E. O., *Nature*, **178**, 542 (1956).

Phytic Acid as a Phosphorus Reservoir in the Developing Potato Tuber

PHYTIC acid is present in the mature potato tuber, as a mixture of dicalcium monomagnesium salts of inositol hexa- and penta-phosphoric acids^{1,2}. The present communication provides information on the effect of maturity and post-harvest storage temperature on the phytic acid content of the potato tuber. Table 1 shows that the phytic acid phosphorus content of freshly harvested tubers increases with increasing maturity, but does not change greatly on subsequent storage at either 25° or 0°. Table 1 also shows that the percentage of total phosphorus present as phytic acid increases with increasing maturity, especially during later stages of development. Thus two-thirds of the increase in phosphorus content per 100 gm. of tuber observed between 80 and 102 days after planting appears as phytic acid phosphorus, whereas at earlier stages of development the incoming phosphorus is preferentially incorporated into starch³. Between two-thirds and four-fifths of the total organically bound acid-soluble phosphorus is present in the tuber as phytic acid phosphorus³. Although the phytic acid contributes a substantial portion of the total phosphorus of the tuber, its concentration in tubers amounts to less than 0.1 per cent of the fresh weight of the tuber.