

compatible with the concept of an electronically conducting layer of adsorbed oxygen on the platinum surface.

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it might be expected to lie between that of the southern⁴ and northern⁵ hemispheres, the rates of increase of which had become parallel about 1958. In 1960 the specific activity of the southern hemisphere atmosphere was rising at about 6 parts per thousand per month and reached $\Delta^{14}\text{C} = 195$ parts per thousand early in 1960 (ref. 4).

It is concluded that the caffeine corresponds to the atmosphere approximately half a year before the growth of the buds. Since the *Camellia thea* plant is some years old (7–90 years) before commercial production, it follows that caffeine is not a metabolic end-product and that it has a metabolic half-life not longer than a few months. This suggests that caffeine is involved in active metabolism.

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BIOCHEMISTRY

Caffeine Metabolism

SOME plants contain organic compounds of potent physiological action on the animal organism; but as far as is known no biological function in the plants themselves. Are they waste end-products of metabolism? Are they the result of detoxification mechanisms? Are they produced to discourage attack by insects and animals? Or are they mere 'accidents' or by-products of faulty metabolism—or perhaps even the results of evolutionary biochemical experiments not yet discarded?

The use, in biological investigations, of carbon-14 produced in nuclear bomb testing has been discussed in earlier communications^{1–3}. We have used this effect to examine the caffeine metabolism in the commercial tea plant (*Camellia thea*). Commercial tea is particularly suitable for such an investigation, since it is derived from buds, less than two weeks old, of a tree many years old.

A sample of commercial tea picked in Ceylon in September 1960 was separated into caffeine and water-extracted leaves. Both the samples were converted to carbon dioxide and counted in the New Zealand Institute of Nuclear Science low-level carbon-14 counter. The ¹²C/¹³C ratio of each sample was measured in a mass spectrometer so that the results could be corrected for any isotope effect in the metabolism of the tree or any possible fractionation in the laboratory procedures. The results are given in Table 1.

Table 1. PARTS PER THOUSAND $\Delta^{14}\text{C}$ VALUES FOR CAFFEINE AND EXTRACTED BUDS OF *Camellia thea*

	Caffeine	Extracted buds
Count rate	100.08	104.05
	$\sigma = 0.35$	$\sigma = 0.36$
$\delta^{13}\text{C}$ with respect to P.D.B. standard (parts per thousand)	-26.6	-25.4
$\Delta^{14}\text{C}$ parts per thousand*	194.6	239.0
	$\sigma = 4.7$	$\sigma = 4.7$

$\Delta^{14}\text{C}$ of atmosphere before nuclear bomb tests = -20 parts per thousand.
 σ = standard deviation of counting.

* $\Delta^{14}\text{C}$ expressed as parts per thousand enrichment above 0.95 of National Bureau of Standards oxalic acid standard and normalized for isotopic fractionation to 0.975 of P. D. B. Chicago belemnite standard.

The caffeine activity is significantly lower than that of the leaves, showing that it was not synthesized in the leaves but that it, or its precursors, were transported into the young leaves.

Although the caffeine is lower in activity than the buds, it is much higher than the atmosphere before nuclear bomb testing ($\Delta^{14}\text{C} = -20$ per cent). No results are available for the change in carbon-14 specific activity of the atmosphere as a function of time at the latitude of Ceylon, but

Separation of Cerebroproteins of Human Brain

THE correlation of protein chemistry with complex functions of the brain has been hampered by the lack of basic information on the brain proteins. Extraction of brain proteins by techniques available so far has yielded usually less than 20 per cent of the total protein content of brain, and only up to 14 components have been demonstrable by electrophoresis¹. Recent work in this laboratory has been directed to the definition of more quantitative methods for the extraction of brain proteins and for more adequate separation, physicochemical characterization, and histological localization by immunochemical methods². We have observed that through a combination of mild buffered extraction, column chromatography on DEAE-cellulose and analysis of the carbohydrate content of each effluent tube, the presence of not less than 50 major chromatographic fractions can be distinguished (Fig. 1). Acrylamide gel-electrophoresis of each of these major fractions shows each to be heterogeneous. Accounting for overlap between chromatographic fractions, a minimum of 100 cerebroproteins from human grey matter have thus been observed. The total yield of protein in these experiments is 6.1 g/100 g wet wt. grey matter—a figure in range for previous figures for total unfractionated brain protein³. The previously known partially characterized protein fractions of brain¹, together with the contribution by serum proteins from blood remaining in small vessels of grey matter, are not likely to account for more than a small percentage of this total yield. Thus, the bulk of the proteins here demonstrated apparently have not been separated or examined previously.

In preliminary experiments 70 g of human grey matter, carefully dissected from autopsy specimens which had been stored at -20°C until extraction, are homogenized either in a Potter-Elvehjem homogenizer (5°C) or in a pre-cooled Waring Blender for 3 min in the cold room, with 100 c.c. of 0.005 M phosphate buffer, pH 7, then centrifuged at 80,000 g for 30 min in a Beckman model L-2 ultracentrifuge. The insoluble residue is rehomogenized with a further 100 c.c. of phosphate buffer and centrifuged, and the second soluble extract combined with the first. The soluble proteins thus obtained (extract A) account for 42 per cent of the total protein recovered.