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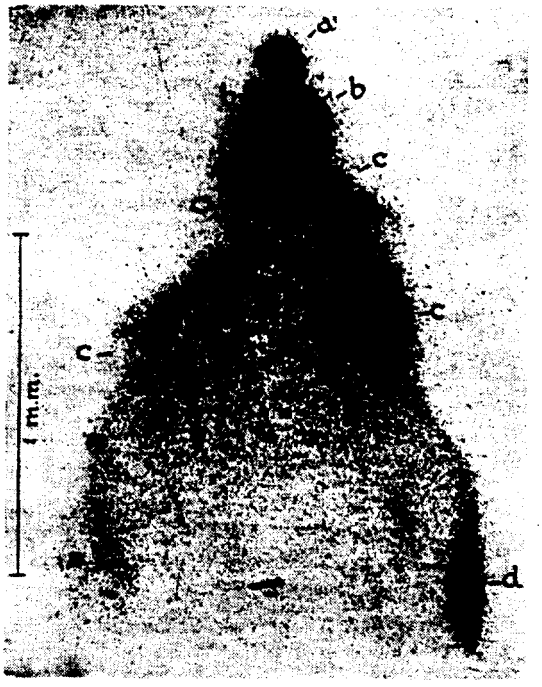
and the phosphate is precipitated *in situ* as the lead salt. The material thus fixed and largely dehydrated is then imbedded in paraffin wax using tertiary butyl alcohol[2] as a solvent and sectioned in the normal way. Radioautographs are then made by the method of Pelc[1].

Since it is necessary to keep the concentration of tracer below a level at which radiation damage is likely to occur[3], thin fine-grain emulsions such as that described by Berriman[4] and Stevens[5] are usually too slow. A faster Kodak experimental radioautographic stripping emulsion has therefore been used. On account of its thickness, this emulsion gives poorer resolution, but points of accumulation of tracer approximately 15 μ apart can be distinguished one from another.



Radioautograph showing distribution of radioactive phosphorus in the stem apex of a young barley plant

The results so far obtainable by this method are illustrated by the accompanying radioautograph of the stem apex of a young barley plant in longitudinal section. The section was 15 μ thick, and the exposure time eighteen days. Concentration of phosphorus occurs especially in the apical meristem (*a*), the leaf primordia (*b*), the bases of the younger leaf sheaths (*c*) and the initial of an adventitious root (*d*). Parts of the vascular tissue also show some accumulation. For the sake of clarity in reproduction, the radioautograph was removed from the section before it was photographed. The most convenient method for locating the site of accumulation of the tracer is, however, to examine the radioautograph and section in contact. Since it is difficult to stain tissues in contact with emulsions, the phase-contrast microscope is useful for this purpose.

Fuller details of the histological and radioautographic techniques will be published elsewhere.

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[1] Pelc, S. R., *Nature*, **160**, 749 (1947).
[2] Johansen, D. A., "Plant Microtechnique" (New York, 1940).
[3] Russell, R. S., and Martin, R. P., *Nature*, **163**, 71 (1949).
[4] Berriman, R. W., *Nature*, **161**, 432 (1948).
[5] Stevens, G. W. W., *Nature*, **161**, 432 (1948).

Regression of a Radioactive Mercurial Diuretic from the Plasma of Man

DURING the study of the mechanism of chronic congestive heart failure and other œdematous states, it became more evident that there is need for a better understanding of the pharmacodynamics of mercurial diuretics. Investigations have been carried out with the use of radioactive mercury incorporated in a mercurial diuretic, 'Mercuhydrin' (sodium salt of methoxyoximercuripropylsuccinylurea with theophylline, prepared in this laboratory by Messrs. H. Krahnke, E. Sprengler and D. Kaestner through the courtesy of Dr. H. L. Daiell of Lakeside Laboratories, Milwaukee, Wisconsin, with the use of mercury-203–205 from the A.E.C. Oak Ridge Operations), in order to observe for a period of 100–180 min. the concentration-time course of the isotope in the plasma of man after intravenous injection.

Fifteen subjects were studied. 2 c.c. of labelled mercurial diuretic was injected into the antecubital vein of one arm of the subjects over a period of about 20 sec., and blood samples were drawn rapidly from the antecubital vein of the contralateral side. The interval between collection of blood samples was 4–8 sec. for the first two or three minutes, thereafter increasing as the experiment progressed. After the blood was centrifuged, 0·3 c.c. of serum was placed upon a filter paper disk, allowed to dry and cemented to a metal disk for counting. Measurements of radioactivity were recorded as counts per minute per cubic centimetre of fluid, and were corrected to values corresponding to an injected activity of 10,000,000 counts per minute of radioactive mercury.

The regression-curves observed in all subjects were similar. The accompanying graph, the composite regression curve of all fifteen subjects, is analysed graphically into three exponential rates of regression.

The regression-rates contributing to the concentration-time course of radioactive mercury in the plasma of man are not simple phenomena; rather they are probably the expression of many simultaneously occurring physico-chemical processes. The most rapid regression-rate is brought about largely by mechanical mixing of the tracer in the plasma along with some extremely rapidly occurring biological events. The second regression-rate probably expresses the filling of the potential mercury spaces and the activation of 'potential turnover patterns' for mercury, including diffusion and chemical reactions, which did not actually exist until a relatively

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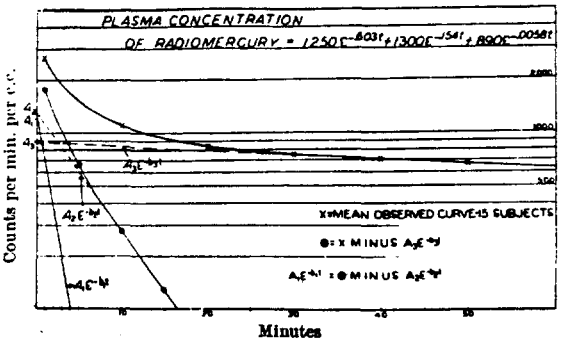

The mean observed plasma concentration-time curve of radiomercury for 15 subjects who received 'Mercuhydrin' intravenously. This curve is analysed graphically into its three exponential regression-rates. The sum of the three individual daughter curves at any time represents the total concentration of radiomercury in the plasma at that time expressed by the general equation, $C_p = A_1e^{-b_1t} + A_2e^{-b_2t} + A_3e^{-b_3t}$

large quantity (78 mgm.) of the element was administered. The third regression-rate is most likely primarily a reflexion of the elimination of the mercury from the body, principally urinary. This is supported by the fact that urinary excretion of radiomercury accounts for practically all this element lost from the plasma after 40 min. or so from the time of injection.

This analysis of the regression of mercury from the plasma of man is undoubtedly an over-simplification of multiple complex phenomena; but it serves as a basis for an understanding of other data dependent upon the concentration of mercury in the plasma. A more detailed discussion of the concentration-time course of radioactive mercury incorporated into a mercurial diuretic will appear elsewhere.

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Significance of the Enzymic Degradation of Cozymase in Living Organisms

REASON for the existence of a very potent enzyme system degrading cozymase in brain[1] and in certain other cells and tissues[2] has been obscure, as intact cozymase is so important to their normal functioning. The rate of breakdown by ground brain is of the order of 1 μmol./mgm. dry weight/hr.[1], whereas the rate of respiration by the intact tissue is about 0·5 μmol./mgm. dry weight/hr. Brain preparations split the cozymase molecule between the pyridine-N and ribose[1]. The results in Table 1 now show that the degrading enzyme can serve cellular economy in the following ways: (1) By decreasing the quantity of specifically the oxidized form of either coenzymes I or II in coenzyme-dihydrocoenzyme mixtures, their oxidation-reduction potentials can be expected to be lowered, and we have found this to be the case experimentally. (2) This can be expected to affect secondarily the many enzymes sensitive to changes in E_H. (3) The degrading enzyme will compete with dehydrogenases for the oxidized forms of the coenzymes, and in mixtures of dehydrogenases favour the action of those of greatest affinity for the coenzymes. (4) Equilibria at certain dehydrogenases can be expected to be modified by the additional presence of an enzyme which removes one component from the system of four which are typically involved. Thus the formation of β-hydroxybutyric acid would be expected to be favoured in the β-hydroxybutyric–acetoacetate system.

Table 1. Substrate specificity of the enzyme-degrading cozymase

Potential substrate (5×10^{-4} M)	Relative rate of degradation
Cozymase	100
Dihydrocozymase	< 0·5
Coenzyme II	65–80
Dihydrocoenzyme II	< 0·5
Cozymase with Dihydrocoenzyme II	90–100

These effects can be expected to influence the course of major reactions in most living systems in which the cozymase-degrading enzyme exists. In agreement with this, considerable changes in carbohydrate metabolism of brain slices have been found[2] to follow inhibition of this reaction by nicotinamide[4], nicotine, 5(4)-3′-pyridylglyoxaline[5] or phenosafranine[6]. The disturbances have included increases (up to ten-fold) in the rates of aerobic or anaerobic glycolysis. Phenosafranine was previously known to inhibit the Pasteur effect in mammalian tissues including brain[6], and some actions of nicotine[6] can be interpreted similarly. The specificity of action of these inhibitors is discussed elsewhere[3]. This evidence for the action of the degrading enzyme in normal tissues implies the existence also of active processes for resynthesis of the coenzymes[7]. The evidence of Judah and Williams-Ashman[8], that linkage between oxidation and phosphorylation in tissue homogenates is inhibited by concentrations of phenosafranine comparable with those of the present experiments, suggests that the degrading enzyme functions in aerobic phosphorylation. Its inhibition, leaving more inorganic phosphate and cozymase available for glycolysis than would otherwise be the case, would thus disturb the Pasteur effect.

Effects of the enzyme from the central nervous system on redox potentials can be very easily demonstrated by experiments of the type illustrated in Table 2. In this, dihydrocozymase to 10^{-4} M was added in Thunberg tubes to solutions containing the dyes of Table 2 in phosphate, pH 6·5, with a 1/30 dilution of a heart extract[9]; half the tubes contained a preparation of the cozymase-degrading enzyme from beef spinal cord (1·2 mgm. dry weight/tube, capable of degrading 3 μmol. cozymase/hr.). The enzyme had no effect on reduction of the positive methylene blue, but caused much greater reduction of the two negative dyes.

Table 2. Effects of cozymase-degrading enzyme on the degree of reduction of dyes by dihydrocozymase

Dyes	Percentage reduced	
	without enzyme	with enzyme
Methylene blue	>97	>97
Neutral red	20	86
Benzyl viologen	< 3	8

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