second sample, 1028-A, was freshly ground. The results agree within our experimental error.

An associated uraninite found with the Besner mine feldspar gave a lead-lead age of $(940 \pm 50) \times 10^6$ yr in agreement with our potassium-argon age (*4*). A. O. Nier has published lead-lead and lead-uranium ages for a uraninite from Blackstone Lake, which is approximately ½ mi from the location of our Conger Township samples (*5, 6*). The mean age of this uraninite is 1010×10^6 yr in good agreement with our potassium-argon ages. Similar results have been obtained by Wasserburg and Hayden (*7*).

These results provide additional support for potassium-argon ages and indicate that the age limits of 800 to 1100 million years assigned to the Grenville orogeny are reasonable (*8*).

References and Notes

1. This investigation was assisted by grants from the National Research Council of Canada and the Geological Survey of Canada.
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Critique of Extracellular Space Measurements with Small Ions; Na^{24} and Br^{82} Spaces

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Perhaps the most generally accepted reference substances for the measurement of extracellular fluid volume are inulin (*1*) and sucrose (*2*), which are thought to be incapable of entering cells, at least in normal circumstances. Since prolonged periods for complete equilibration in extracellular fluid are required, owing to the relatively large molecular size and slow diffusion rates of these substances in tissue, the search continues for other agents that might permit more rapid equilibration and simpler experimental procedure. Both sodium and bromide are known to penetrate cells to a certain extent, and the space of distribution of Na^{24} 3 hr following intravenous administration (*3*) is significantly in excess of that now believed to represent the volume of the extracellular compartment. However, it was thought that intracellular permeation might be sufficiently slow compared with extracellular diffusion to permit evaluation of the extracellular compartment.

The concentration-time course of radioactivity in plasma, following intravenous administration of Na^{24} or Br^{82} to human subjects, is a multicomponent curve. The concentration in terms of the fraction of the total radioactivity in the body per liter of plasma (Fig. 1*A*) becomes virtually constant after about 12 hr in

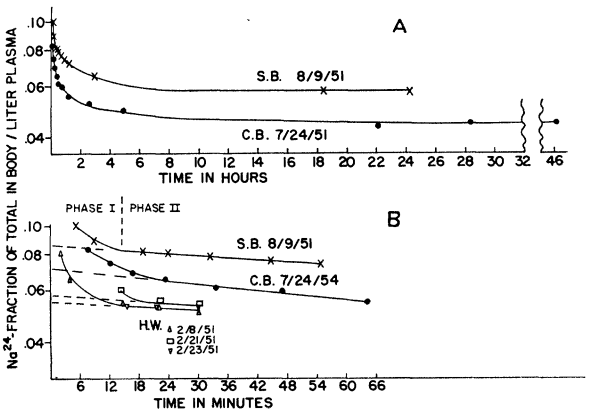

Fig. 1. Venous plasma curves. Phase I is related chiefly to mixing in extracellular fluids.

normal subjects. Between about 15 to 20 min and 1 hr (phase II), the concentration decreases almost exponentially (Fig. 1*B*) with a much shallower slope than during the initial period (phase I). It seemed reasonable to suppose that phase I may reflect, primarily, mixing in extracellular fluid and that phase II is chiefly attributable to penetration of cells and (in the case of Na^{24}) bone apatite. Then the extracellular fluid volume would be given approximately by the reciprocal of the zero time extrapolation of phase II, assuming that intracellular penetration proceeded at the same rate throughout phase I as during phase II.

This assumption introduces at least slight errors into the calculations, since these ions cannot penetrate most tissue cells until they have diffused through some part of the extracellular compartment. In addition, errors of unknown magnitude may result from very rapid penetration of some cells during the period of extracellular diffusion. Entrance of Na^{24} into erythrocytes is negligible at this time, but equilibration of Br^{82} between erythrocytes and plasma occurs almost instantaneously and can be corrected for by simultaneous measurement of total red cell volume. Negligible amounts (½ percent or less) of either ion are excreted during the first ½ hr.

The calculations just described have yielded values for extracellular space in nonedematous subjects which are reproducible (H.W., Fig. 1*B*) and are in good agreement with those obtained by inulin or sucrose—that is, about 13 to 19 percent of body weight (Table 1). It was further observed that simultaneous space measurements with Na^{24} and Br^{82} (Fig. 2) give almost identical values when correction is made for Br^{82} in erythrocytes (Table 2). However, certain observations demonstrate the unreliability of these estimates of extracellular fluid in patients with large extracellular fluid collections such as edema, ascites, and pleural effusion. The specific activities in the abnormal fluid

Table 1. Extrapolated early Na^{24} spaces in nonedematous subjects.

Subject	Diagnosis	'Extracellular' space* (% body wt.)
S.B.	Diabetes	17.3
F.B	Rheumatoid arthritis	13.9
H.W.	Compensated cirrhosis	16.7
B.G.	No disease	16.5
A.C.	Polycythemia	17.4
S.B.	No disease	13.8
C.B.	Uretero-sigmoidostomy	18.2

* Corrected for plasma water and the Gibbs-Donnan factor.

Table 2. Extrapolated early Na^{24} and Br^{82} spaces* (percentage of body weight).

Subject†	Na^{24}	Br^{82}	Na^{24}/Br^{82}
S.K.	19.4	20.1	0.965
D.L.	26.2	27.0	.972
D.D.	18.5	18.1	1.02
K.A.	21.1	21.1	1.00

* Corrected for plasma water and the Gibbs-Donnan factors. Br^{82} spaces have also been corrected for red cell penetration.
† Nonedematous subjects with malignancies. High values for the spaces relative to body weight are related to marked weight loss.

collections may not reach that of the plasma for several hours (Fig. 3), indicating that equilibration in these extracellular fluid spaces is far from complete at the time of onset of phase II. Kaltreider and associates (*3*) noted that pleural fluid equilibration may not be complete in some cases even after 3 to 6 hr. Thus the value derived from extrapolation of the slow component seriously underestimates the true extracellular space in these cases. Although it is virtually impossible to obtain satisfactory samples of extracellular fluid in nonedematous subjects, the concentration curve of thoracic duct lymph (Fig. 3) indicates that, in some extracellular sites at least, equilibration is not complete by the time of onset of phase II. This is almost certainly also true of tendon and similar tissues, in which extracellular diffusion equilibrium is prolonged (*4*).

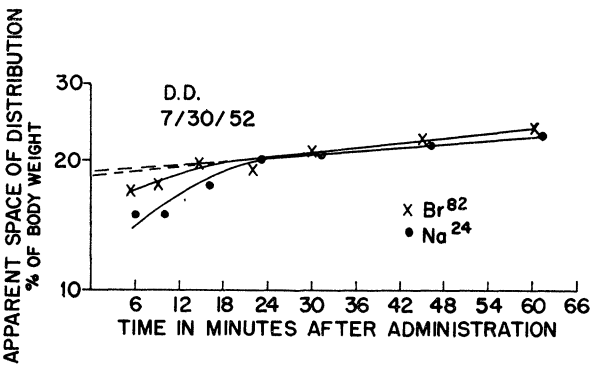

Fig. 2. Spaces of distribution of Na^{24} and Br^{82}. Spaces were calculated from the venous plasma concentrations, correcting for erythrocyte penetration of Br^{82} and for plasma water and Gibbs-Donnan factors.

It appears likely that in nonedematous subjects all small freely diffusible ions distribute into a large fraction of the extracellular compartment within 15 to 20 min or so. However, extracellular space is not completely equilibrated at this time, and some intracellular sites have probably also been penetrated. Although the apparent space of distribution may then fairly well approximate the true extracellular space,

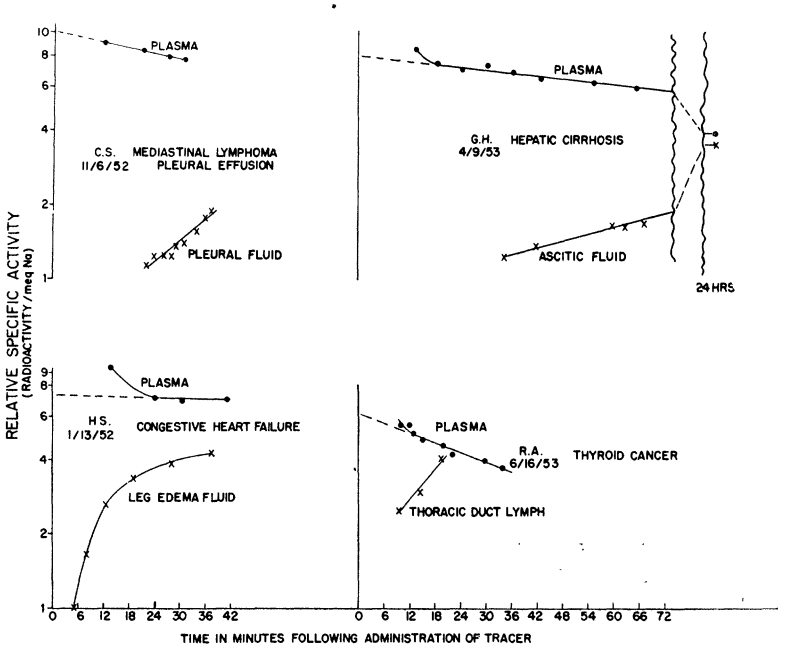

Fig. 3 Relative specific activities of venous plasma and extracellular fluids as a function of time following intravenous administration of Na^{24}.

this value is clearly not to be relied upon as a precise measure of extracellular fluid. Where the extracellular fluid is markedly increased, such measurements are grossly in error.

Thiosulfate (*5, 6*) and radiosulfate (*7, 8*) have been suggested as useful for the measurement of extracellular space. However, the criticisms that have been directed against Na^{24} and Br^{82} apply with the same force to any other ions that penetrate cells or undergo metabolism. The studies reported with radiosulfate and thiosulfate have failed to demonstrate either complete equilibration in the various extracellular compartments or the absence of cell penetration at the time of measurement. They have also failed to take sufficient account of the prolonged period of equilibration required in the presence of an expanded extracellular compartment. Thiosulfate, particularly, has additional disadvantages. The exponential segment of the thiosulfate plasma curve has such an extremely sharp slope, in consequence of its rapid metabolism and excretion by the kidneys, that large errors in the ordinate intercept of the extrapolated line result from small errors in the experimental observations and from the invalidity of the assumption of a constant rate of intracellular penetration. Furthermore, it appears from the published figures (*5*) that the exponential segment of the plasma curve (phase II) may begin as early as 2 min after the end of an 8-min infusion of thiosulfate. Since thiosulfate has a smaller diffusion coefficient than Na^{24} and Br^{82}, it is difficult to accept complete extracellular equilibration of thiosulfate in this short time, and it may be concluded that measurements with this ion are even less reliable than those utilizing Na^{24} and Br^{82}, which are at least free from the objections of rapid metabolism and excretion.

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Melanophore-Contracting Hormone (MCH) of Possible Hypothalamic Origin in the Catfish, *Parasilurus*

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Although it is well established that the pituitary is the source of a melanophore-expanding hormone (intermedin), there is no conclusive evidence for the existence of another kind of pigmentary hormone antagonistic to intermedin (W-substance of Hogben). There are only indirect indications, such as the observation that pigment concentration in the melanophores is disturbed in the absence of the pars tuberalis in amphibians or of the pars distalis in elasmobranchs. The effect of injection of extracts or of implantation of possible sources of the presumed melanophore-contracting hormone has as yet not been adequately studied (*1*).

In the present investigation it was found that crude aqueous extracts prepared from the hypothalamus and from the pituitary of the oriental catfish, *Parasilurus asotus,* contain, in addition to intermedin, a hormone principle responsible for melanophore contraction. This principle is tentatively designated as

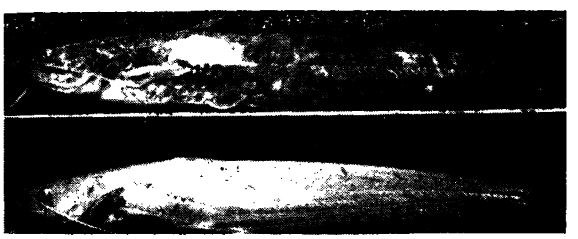

Fig. 1. Catfishes showing the effect of injection of hypothalamic extracts: (top) local effect of crude aqueous extracts; (bottom) pronounced effect of concentrated alcohol-insoluble fraction.

MCH (melanophore-contracting hormone). The extracts mentioned, when injected into "black adapted" hypophysectomized catfish with approximately intermediate pigment dispersion (*2*), caused a marked but localized pallor at the site of injection and simultaneously a considerable darkening of the rest of the body. Highly concentrated extracts resulted in increased blanching, but generalized pallor was rarely observed following the injection (Fig. 1). Administration of fractions obtained by treatment of the extracts with absolute ethanol showed that the alcohol-insoluble fraction had a higher MCH content, being comparatively free of the antagonistic intermedin which was concentrated in the alcohol-soluble fraction. In pieces of skin kept *in vitro,* the melanophores that had been made to expand under bright light responded well to the MCH fraction of the extracts. As a result of dilution experiments, a measure for MCH activity was determined in such a way that the potency of an extract which was sufficient to induce a state of maximal melanophore contraction *in vitro* in 15 min at 20°C was designated as one *Parasilurus* unit (PU). A comparison of the relative effect, in terms of PU, of hypothalamus and pituitary extracts showed that the concentration of MCH in the pituitary was approximately 4 or 5 times that of the hypothalamus. In the pituitary, the hormone was found in highest concentration in what is called "Übergangsteil" (*3*), namely, the characteristic component of certain teleost pituitaries situated between the so-called anterior lobe and the neuro-intermediate lobe.