what denser blood as compared to the alkaline saline standard. Red cells were prevented from settling in the whole blood samples by manual agitation with wire loops prior to the counting. The cells were hemolyzed with saponin in the standard solution. The results (Fig. 1) show close superimposition of the values indicating near equality of the volumes of distribution of these 2 agents.

Summary. The use of the well-type sodium iodide crystal scintillation counter to measure P^{32} and I^{131} from a single sample of whole blood is described. The individual activities are calculated from data obtained with and without a metal cup absorber.

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Phosphate (P^{32}) Exchange in Brain Phospholipids *in vivo* and *in vitro*.*† (20823)

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It is now well established that adult neural tissue can synthesize phospholipids *in vitro* as well as *in vivo*(1-7), and the inference that these "structural" components of the cell are involved in normal functional metabolism has some direct support(6-8). On the other hand, the slow phosphorus turnover of brain phospholipids would preclude a very active metabolism unless the apparent low overall specific activity in fact represents high activity of a small fraction. In favor of this, it had been shown(1,2) that P^{32} is incorporated to a greater extent into brain cephalins and sphingomyelin than into lecithins; and in *in vitro* studies we observed much higher specific activities in some lipid fractions than in others. Further study shows a highly active protein moiety (derived from lipoprotein) which may represent a part of the inositol metadiphosphate complex isolated by Folch and Lees(9).

Methods. In vitro, the brains of 6 to 12 male Sprague-Dawley rats were used in each experiment. Each brain was homogenized in 8.0 cc of 0.01 M potassium phosphate buffer (pH 7.4) immediately after it was removed from the skull and kept at −7°C until the brain tissues of the different animals were combined. The entire homogenate, together with $Na_2HP^{32}O_4$, glucose (10^{-1} M), K ATP (3.1×10^{-4} M), DPN (6.1×10^{-5} M), cytochrome c (5×10^{-5} M), $MgCl_2$ (8.0×10^{-3} M), and KF (8.0×10^{-3} M) was incubated at 37°C in the Warburg water bath. After shaking for 20 minutes, one hour, or 2 hours, the tissue was separated from the incubating medium by centrifugation at −7°C for 20 minutes at an R.C.F. of 2700 times gravity. The lipids were extracted from the brain tissue at 4°C with 20 volumes of cold chloroform-methanol (2:1) per g of tissue(9), and the extract was separated from the residue by filtration at 4°C. The residue was reextracted 2 more times, the 3 filtrates were combined, and were washed several times with distilled water at 4°C by the method of Folch(9) to remove water-soluble contaminants. The chloroform-methanol extract was then dried *in vacuo*. This process released some of the protein from lipoprotein elements which were soluble in chloroform-methanol(9).

The lipids were redissolved in 20 volumes

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TABLE I. Specific Activities of Various Fractions after Incubation of Brain Homogenates with P^{32} at 37°C.

Time of incubation	Inorganic phosphate*	Delta 7*	Organic acid-soluble*	Lipid extract	Lipid / Delta 7
20 min.	1188	929	753	1.1	1.2×10^{-3}
1 hr	1132	972		1.1	1.1 "
2 "	1124	945	737	1.2	1.3 "

* Brain tissue was separated from medium by centrifugation. These fractions were isolated from supernatant.

TABLE II. Specific Activities of Various Crude Fractions Obtained from Lipid Extracts of Brain Tissue.

Fraction	% total PL-P	*In vitro*. Separation of protein: Optimal	Partial	Omitted	*In vivo*
Inorganic phosphate of medium		1025			
Entire lipid extract*	100	1.0	1.0	1.0	1.0
Protein	1–2	20.4	13.0		.77
Acetone soluble	1–2	.25	.84		
Methanol "	88–95	.20	.62	.55	.92
Ether "	2–6	5.32	6.19	9.48	.73
Chloroform-methanol soluble	0.5–3	2.71	8.07	17.4	.99

* Specific activity of entire lipid extract designated as unity.

of chloroform-methanol and separated from the insoluble protein components by filtration at 4°C. The protein moieties were washed 6 to 10 times with a total of over 100 volumes of chloroform-methanol to remove adhering lipids. The lipids which had been redissolved were dried *in vacuo* again. The dry residue was then extracted at 0°C 5 times with a total of 100 volumes of each of the following solvents: acetone, followed by 95% ethyl alcohol, and finally diethyl ether. The residue was redissolved in chloroform-methanol. Although each of these fractions contains a mixture of substances(10), the choline lipids are found predominantly in the methanol extract, whereas the cephalins are soluble, for the most part, in ether and chloroform. The specific activities (counts/min./μg of phosphorus) of the entire extract and of the different fractions were measured after ashing in 10 N H_2SO_4 for 12 hours at 110°C. The exchange of P^{32} in the acid-soluble lipoproteins was not investigated. In one experiment, the specific activity of the phosphate of the incubating medium was measured. Inorganic and delta 7 phosphates were precipitated as $MgNH_4PO_4$, and the remaining "organic acid-soluble" phosphate (representing mainly the phosphate of the glycolytic intermediates) was obtained by ashing at 110°C in 10 N H_2SO_4 for 12 hours. The specific activities of these components were ascertained after incubation times of 20 minutes, one hour, and 2 hours. Also, in one experiment, sphingomyelin was isolated according to the method of Brante(10).

In vivo, 375 μc of P^{32} were injected intraperitoneally into each of 4 rats, and the animals were sacrificed 20 hours later. The lipids obtained from the brains of these animals by homogenization in chloroform-methanol were washed and divided into crude fractions as described above. In other experiments, rats were injected with 200 μc of P^{32} and sacrificed 20 minutes, one hour, 4 hours, and 20 hours later. In this case, the lipids were isolated in a single fraction by the method of Schneider (11) and the specific activity of the entire extract was measured.

Results. In vitro, the total phospholipids as well as the acid-soluble components of the medium reached their maximum specific activities within 20 minutes after the start of incubation and then held constant through the 2-hour period (Table I). The lipid extract attained a specific activity approximately 1/1000 that of the inorganic phosphate of the medium.

TABLE III. Specific Activities of Lipid and Delta 7 Fractions of Normal Rat Brain at Various Times after Intraperitoneal Injection of 200 Microcuries of P^{32} per Animal.

Time	No. animals	Lipid (±1 S.D.)	Delta 7 (±1 S.D.)	Lipid / Delta 7
20 min.	3	.057 (±.018)	21.3 (± 4.3)	2.6×10^{-3}
1 hr	3	.24 (±.016)	39.3 (± 7.1)	6.1×10^{-3}
4 "	3	.95 (±.26)	34.6 (± 4.0)	2.7×10^{-2}
20 "	2	3.0 (±.0)	29.8 (± 2.6)	1.0×10^{-1}

In other experiments the lipids were divided into crude fractions as described above. Taking the specific activity of the entire phospholipid extract as unity, values of the fractions varied from 20.4 for the protein to 0.2 for the methanol portion—a 100-fold range (Table II). Sphingomyelin, actually isolated from the lipid fraction, had a specific activity of only 0.09 on this scale; and, conversely, when the preliminary separation of the protein was less complete, or was omitted, its activity appeared mostly in the chloroform-methanol fraction (Table II).

In vivo, by contrast, the specific activity of the total phospholipids rose continuously for the 20-hour period (roughly linearly during the first few hours after the intraperitoneal administration of P^{32} (Table III), and a considerably greater amount of P^{32} was eventually incorporated *in vivo* than *in vitro* (see also(4)); approximately 80 times as much after 20 hours, if it is assumed that the delta 7 fraction comprises the source of the phospholipid phosphate. Moreover, all fractions showed approximately the same specific activity, the range being 0.73 to 0.99 (Table II).

Discussion. Two points require attention in comparing *in vivo* and *in vitro* results: 1) the much reduced turnover *in vitro*, both in time to equilibrium and in total; and 2) the great quantitative differences in turnover between fractions *in vitro* but not *in vivo*. A loss of general synthetic power *in vitro* is understandable as a result of the limited availability of adenosine triphosphate(14), dilution of reactants, enzyme destruction(3), or activation of autolytic reactions(12) consequent to the disruption of structure. For example, the relative excess of breakdown to synthesis in brain homogenates decreases the total lipid content by 10% to 15% after several hours incubation(4,13). The second point, that *in vitro* turnover is perhaps limited to one or 2 "trace" components of the total phospholipids, can be interpreted along similar lines if it be assumed that the active substances normally serve as phosphate carriers to the other phospholipids. *In vivo* all steps in the sequence are intact and the specific activities rise more or less together; *in vitro* the lipoprotein step primarily remains, with the results observed.

In *in vitro* experiments it was observed that the "active" material of the protein moiety was predominantly chloroform soluble. Since the protein fraction actually contains diphosphoinositide(15) which is soluble almost exclusively in wet chloroform, it is possible that this substance contains most of the P^{32} incorporated into the protein fraction. However, the relatively high specific activity of the ether soluble material indicates that other cephalins are also involved in phosphate exchange *in vitro*.

Summary. *In vitro*, the specific activity of the phospholipid phosphate reaches a maximum within 20 minutes after the start of incubation, while *in vivo* the specific activity of this fraction rises continuously for at least 20 hours after the intraperitoneal administration of P^{32}. When the lipid extract is divided into crude fractions, it is observed in *in vitro* experiments that the specific activities of these fractions are markedly dissimilar. Conversely, the specific activities of these fractions are approximately the same when obtained from the brains of rats that had been injected with P^{32} 20 hours previously. Some of the factors involved in these findings have been discussed.

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Ultra-microdetermination of Arginine by a Compound Microbiological Assay Method.* (20824)

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Most microbiological assay methods are based on the principle that an assay organism grows and produces acid proportionately to the concentration of an essential nutrient added at different levels to a basal medium lacking this substance but otherwise nutritionally complete. In amino acid assays the ratios between the moles of acid produced and nutrient added range from about 600:1 (glutamic acid) to 11,000:1 (tryptophan). Magnification in response evidenced by such ratios is a general characteristic of titrimetric microbiological assays.

From unpublished studies in this laboratory it became apparent that the magnification in response inherent in a given microbiological assay could be compounded by means of a 2-stage process in which the growth of a primary organism is regulated by the level of an essential nutrient added to the basal medium and that of a secondary organism by the level of an essential nutrient introduced into the basal medium as a metabolic product of the primary organism.

The determination of an amino acid by a compound microbiological assay procedure is described for the first time in this report. *Lactobacillus casei* 280-16 was selected as the secondary assay organism because of its response to D-lactic acid, a metabolic product of many assay organisms(1). The anticipated gain in sensitivity with this secondary assay was about 2000-fold since the quantity of L-lactic acid produced (available for titration) by *L. casei* 280-16 has been shown to be about 2000 times that of the D-lactic acid available for the growth of this organism(1). *Lactobacillus casei* ϵ (American Type Culture Collection No. 7469) was selected as the primary assay organism because it produces only relatively small amounts of D-lactic acid (1). The resulting relatively low effective magnifications (ratios between D-lactic acid produced and nutrient added) in primary assays with this organism seemed desirable in preliminary studies to avoid difficulties which might be anticipated in compound assays with the highest over-all magnifications. Arginine was selected as the test amino acid since it may be determined satisfactorily in biological materials by a standard (one-stage) assay with *L. casei* ϵ(2).

The compound microbiological assay method described in the present report for the determination of arginine with *L. casei* ϵ and *L. casei* 280-16 in combination has proved to be approximately 140 times as sensitive as the standard procedure while retaining the advantage of conventional macroscale manipulations.† Since other organisms commonly employed in standard assays produce proportionately much more D-lactic acid than

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