tin. The quantitative precipitin curves obtained with rabbit anti-ferritin and ferritin are characterized by a sharp rise in N precipitated by increasing addition of N in the antibody excess zone, and a very slow decline in N precipitated in the region of increasing antigen excess. This type of curve, previously described(2) and confirmed in these experiments, makes determination of the zone of equivalence more difficult. The quantitative isotope precipitin curve provides an advantageous method of antibody determination with chicken or rabbit anti-ferritin systems since the anti-ferritin content of a serum can be expressed as μg N of antigen precipitated per ml of antiserum.

Chicken anti-ferritin contains 3 immunoglobulins which react with ferritin in precipitin arcs, demonstrated by iron staining or $I^{131}F$ autoradiography. This heterogeneity of chicken antisera has been previously described (8) in the chicken anti-insulin system. Attempts to separate these antibodies for further characterization are in progress. The use of iron staining of complexes of anti-ferritin immunoglobulins and ferritin provides a method of study of antibodies similar to autoradiographic immunoelectrophoresis without involving the use of radioactive labeled antigens or antibodies.

Summary. Gel filtration was used to obtain a preparation of horse spleen ferritin apparently free of antigenic contaminants. The purified ferritin was labeled with I^{131}. Quantitative isotope precipitin curves with rabbit and chicken anti-ferritin are described. The staining of ferritin iron in ferritin reacting with immunoglobulins of electrophoretic preparations was used to show the heterogeneity of chicken antibodies in the primary and secondary response against ferritin.

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Absorption, Distribution and Excretion of Pentaerythritol and Pentaerythritol Tetranitrate by Mice. (29829)

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Although pentaerythritol tetranitrate (PETN) has been used extensively for many years to prevent angina pectoris, there is a dearth of information about its metabolism. One of the difficulties has been the lack of assay methods for the intact PETN molecule. Previous investigators demonstrated PETN absorption by the difference between the dose administered to rats and the subsequent nitrate content of the gastrointestinal tract (1) and by nitrate and nitrite assays of the blood of humans who ingested the drug (2,3,4). Since these approaches omit the intermediary metabolism of PETN, they preclude furtherance of our interest in elucidating the mechanism of action of this drug.

To this end methods were developed for qualitative and quantitative determination of PETN and its hydrolysis products(5). The application of these procedures to the fate of PE and PETN in the mouse is reported here.

Materials and methods. The mice employed were 20-24 g females, Swiss Ha/ICR strain supplied by Millerton Research Farm, Millerton, N. Y. C^{14}-PE (labeled at C1 and C2) was synthesized from acetaldehyde with specific activity of 1.6 millicuries/millimole and was employed to prepare C^{14}-PETN (m. pt., 140-141.5°). To minimize the danger of working with explosive material, the labeled PETN was mixed with 7 parts by weight of C.P. lactose. The activity of the lactose-C^{14}-PETN mixture was 0.59 millicurie/g.

PETN study. Mice were given 10 mg/kg of C^{14}-PETN by gavage. Then the mice were water-loaded by injecting 2 ml of water subcutaneously into the ventral abdominal region of each mouse. Pairs of mice were kept in glass metabolic units for 15 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, 72 hours and 96 hours. While housed, the mice received neither food nor water. Mice kept more than 1 day were force fed 1 g of feed as a paste and water loaded daily. The urine excreted by groups of 6 mice was filtered and pooled. Expired air was bubbled through alkali for 24 hours to trap carbon dioxide. Blood samples were drawn from the same mice before sacrifice. All fecal material from the first formed pellet to the anus was collected and combined with defecated matter including that filtered from the urine. A number of internal organs, fat pads from the lumbar region, and the remaining cadavers were also pooled by group.

The radioactivity of each urine sample was determined by mixing 1.0 ml urine with 19.0 ml of scintillation fluid in a counting vial and assaying in a Packard Tri Carb spectrometer at 7°C. The scintillation solution consisted of 7.0 g of 2, 5-diphenyloxazole, 0.3 g of 1, 4-bis-2-(4-methyl-5-phenyloxazole)-benzene and 100 g of naphthalene in 1.0 l of freshly distilled dioxane. An internal standard was used to evaluate the quenching characteristics of each sample.

TABLE I. Thin Layer Chromatography of PE and Its Nitrates.

Reference compound	R_f value Solvent A*	Solvent B*
PE	.00	.00
PE-Mononitrate	.05	.35
PE-Dinitrate	.20	.70
PE-Trinitrate	.50	.80
PETN	.70	.85

* Solvent A: toluene + ethyl acetate, 1:1, v/v.
Solvent B: ethyl acetate saturated with water.

The qualitative analysis of urine samples for PETN and its metabolites was conducted by thin layer chromatography as described previously(5). Essentially, the assay consists of (a) spotting aliquots on 2″ × 8″ glass plates coated with 300 μ of silica gel G bound with $CaSO_4$, (b) development of the chromatograms with the ascending technique, (c) drying the plates, (d) scanning the plates for radioactive areas with a Nuclear Chicago Actigraph II equipped with an adapter for thin layer chromatography, and (e) determining the area under each peak with a compensating polar planimeter. The solvent systems employed were toluene-ethyl acetate (1:1, v/v) and ethyl acetate saturated with water.

The fat pools were homogenized and extracted with ether. Aliquots of the ether extracts were employed for thin layer chromatography and scintillation counting. Each of the other tissue pools and fecal pool was homogenized and shaken overnight with 40 ml of water. After the mixture was centrifuged, aliquots of the supernatant solution were assayed by scintillation spectrometry and by thin layer chromatography as above. The water-insoluble residue was extracted by shaking overnight with 20 ml of acetone. The mixture was centrifuged and the supernatant solution was evaporated to dryness. The residue of acetone-soluble material was dissolved in dioxane and submitted to quantitative and qualitative assay.

PE study. A single dose of C^{14}-PE (10 mg/kg) was administered orally to 12 mice. Each animal was water-loaded as described above and placed into a separate glass metabolic unit. At intervals of 15 minutes, 4

TABLE II. Recovery and Identity of C^{14} Compounds After Oral Administration of C^{14}-PETN to Mice.

Sample	% of administered dose recovered															
	15 Min		1 Hr		2 Hr		4 Hr		24 Hr		48 Hr		72 Hr		96 Hr	
	PE	PETN	PE	PETN	PE	PETN	PE	PETN	PE	PETN	PE	PETN	PE	PETN	PE	PETN
Urine	.0	.0	.3	.0	14.3	.0	22.3	.0	36.3	.0	45.0	.0	58.0	.0	57.6*	.0
Feces	.1	.0	.1	.0	3.5	.0	2.1	.0	5.0	.7	21.1	.8	8.8	1.2	13.7	1.7
GIT	.0	95.4	.0	82.7	.0	64.9	.0	51.7	8.2	.5	.78	.06	.20	.02	.05	.00

* In this group, excretion of PE was 42.8% after 24 hr, 50.0% after 48 hr, 53.1% after 72 hr, and 57.6% after 96 hr.

TABLE III. Recovery of C^{14}-PE After Oral Administration to Mice.

Sample	% C^{14}-PE recovered		
	15 Min	4 Hr	24 Hr
Urine	.0	44.3	53.1
Feces	.0	23.8	36.1
GIT	54.1	13.2	3.1

hours and 24 hours, blood samples were drawn from groups of 4 mice which were subsequently sacrificed. Urine, feces, lumbar fat pads, various internal organs and the remaining cadavers were pooled appropriately. Urine samples were assayed directly, both quantitatively by scintillation spectrometry and qualitatively by thin layer chromatography. The fat pools were extracted with ether for the same purposes. Each of the other pooled materials was homogenized in 40 ml of water and extracted by shaking for 24 hours before assay by the same precedures.

Results. Table I shows the Rf values of PE and its 4 nitrates obtained by thin layer chromatography in 2 solvent systems. Table II presents the results of the PETN absorption study. It is evident that PETN was absorbed slowly; approximately half of the dose left the gastrointestinal tract in 4 hours. The excretion rate was also slow; 3-4 days were required for the passage of 58% of the C^{14} into the urine. Thin layer chromatography indicated that PETN was excreted entirely as PE. The present state of development of methodology did not permit identification of the C^{14} compounds present in blood and in most of the tissues.

PE was absorbed and excreted rapidly (Table III). Almost half of the administered dose left the gastrointestinal tract within 15 minutes and 68% of the dose appeared in the urine and feces after 4 hours. The only compound detected was PE.

A comparison of the distribution of PE and PETN appears in Table IV. It became increasingly difficult with time to recover C^{14} administered to mice as PETN; only 60% of the radioactivity was recovered at 24 hours postadministration. Very little (0.1%) of the C^{14}-PETN was converted into respiratory carbon dioxide over this period.

Discussion. It is evident from Table I

TABLE IV. Distribution of C^{14} in Mice Given PE and PETN.

	% of C^{14} administered					
	15 Min		4 Hr		24 Hr	
Sample	PE group	PETN group	PE group	PETN group	PE group	PETN group
Blood*	.5	.1	.03	.1	.01	.1
Brain	.1	.04	.1	.02	.01	.42
Fat†	3.7	.5	1.0	3.5	.7	2.5
GIT	54.1	95.4	13.2	51.7	3.1	8.7
Heart	.2	.01	.02	.02	.02	.01
Kidney	1.1	.2	.6	.1	.3	.3
Liver	4.6	.7	1.5	.5	.8	.8
Lungs	.4	.05	.1	.02	.2	.1
Spleen	.2	.00	.5	.05	.1	.01
Carcass	21.6	3.1	13.4	2.4	3.5	6.8
Urine	.0	.0	44.3	22.3	53.1	36.3
Feces	.0	.1	23.8	2.1	36.1	5.7
Expired air	—	—	—	—	—	.1
Total recovery	86.5	100.2	98.6	82.8	97.9	61.8

* Blood was assumed to constitute 10% of body wt.
† Fat was assumed to constitute 12% of body wt.

that thin layer chromatography in both solvent systems is needed to resolve all of the 5 compounds under consideration. This situation is not especially disadvantageous because the use of the second solvent provides corroboration. The problem with the identification of PETN metabolites in blood and in tissues is not only the low concentrations, but the binding of the tagged compounds to extracted tissue substances. Such combinations were observed to migrate as the tissue component during thin layer chromatography. We are now investigating possible means of liberating the metabolites without artifactitious degradation of PE-nitrates.

Carr and Krantz(6) pointed out that polyhydric alcohols containing 2, 4 or 7 hydroxyl groups generally are not transformed into glycogen and are excreted unchanged. Subsequently, Kutscher(7) reported that humans who ingested PE excreted 85-87% of the unaltered compound in the urine after 30 hours. The present study also substantiates the generalization of Carr and Krantz. The mouse rapidly absorbs PE and excretes it unchanged. We consider that the PE found in feces resulted from urine soaking the feces due to the design of the metabolic units employed.

Likewise, it seems that the PE in the feces of mice administered PETN is attributable to contamination with urine. The PE found in the gastrointestinal tract of these animals is considered to have resulted from coprophagy rather than from the hydrolysis of PETN in the stomach. The absence of the 3 intermediate PE-nitrates in the gastrointestinal tract at any time supports this view, particularly since a study conducted *in vitro* (5) indicated that the 4 nitrate esters of PE undergo hydrolysis in acid at approximately the same rate.

The results of the present study differ greatly from the report of Lawton *et al*(1) to the effect that "Six hours after feeding, PETN can be recovered almost entirely from the alimentary canal of white rats." As stated above, Lawton *et al* did not assay directly for PETN, but relied upon nitrate determinations. It seems probable that the disparate conclusions of the two studies are due to this indirect assay rather than to a species difference between the mouse and the rat.

Summary. Radioactive PE and PETN were administered orally to mice and their fate was followed by scintillation spectrometry, thin layer chromatography and radioscanning. PE was absorbed rapidly and excreted unchanged whereas PETN was absorbed slowly and excreted as PE.

The authors appreciate the contribution of Mr. Edward Merrill who synthesized the radioactive compounds and also determined the C^{14}-CO_2 of air exhaled by mice given C^{14}-PETN.

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In vitro Incorporation of Radiophosphorus into the Phosphatides of Normal Human Blood Cells.* (29830)

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In the present study, normal human blood was incubated with P^{32} labeled orthophosphate (P^{32}), the cells separated, and quantitative measurements of P^{32} incorporation into various phosphatides of red cells, leukocytes and platelets were made. Previous reports of measurements of the turnover of the phospholipid, phosphatidic acid, in the red cell have not shown consistent results(1-4). Similarly, the *in vitro* activity of leukocyte and platelet phosphatides, as measured by P^{32} uptake, differs in normal cells separated prior to incubation(5,6), in leukemic WBC(7) and in WBC obtained by peritoneal irritation(8). Since phosphatide activity in normal circulating whole blood cells, not separated prior to incubation, might also vary, this could partially explain the inconsistency of results. Values for normal cells similarly prepared would be useful for comparison to leukemic WBC and those obtained by peritoneal stimulation.

Methods. Fresh human blood anticoagulated with heparin or Na_2 EDTA was mixed with a solution containing 15 μc of high specific activity $Na_2HP^{32}O_4$ (or 30 μc acetate-1-C^{14}) and 1 mg glucose per ml packed RBC. The pH was maintained at 7.30 $\pm$ 0.10 by adjusting the flow of 5% CO_2 in air through the mixture. After constant shaking at 37°C for the desired time, aliquots were removed and erythrocyte, leukocyte or platelet concentrates prepared. Samples were obtained at regular intervals in studies in which the rate of incorporation of P^{32} was being determined and at 3.2 hours for all studies in which uptake into the various cell types was being compared. To obtain *RBC fractions,* samples of whole blood were thrice centrifuged and washed with 0.9% NaCl with removal of the buffy coat after each centrifugation. Less than 1 WBC and 2 platelets per 5×10^3 RBC were observed. *Leukocytes* were obtained by sedimentation of whole blood with 3% dextran (Mol. Wt. 200,000-275,000, Sigma) in 0.9% NaCl at 4°C. The sediment was washed thrice with 0.9% NaCl. These fractions contained cell ratios which ranged between 3-16 RBC/20-25 platelets/1 WBC. Calculations were corrected for contaminating red cells and platelets. *Platelets* were obtained by differential centrifugation. The platelet rich sediment was washed 3 times with 0.9% NaCl. Leukocytes and red cells were not seen during the platelet counts. Erythrocyte and WBC counts were performed by use of an electronic cell counter, and platelet counts were done with phase microscopy. Additional counts and observations were made on blood films after staining with Wright's stain.

Extraction of the lipids of all cell types was accomplished by previously described techniques(9). Phosphorus was determined by the method of Bartlett(10). Column chromatographic separation of phospholipids was performed as modified by Chang and Swee-

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