is corrected (x 3.3) to dry weight one figure is 39 mg/g(12). This agrees with normal SAE values obtained by more sensitive methods in the present series. It is clear that many other lipid analyses, in common with these data, are to be regarded as first-order approximations, of greater value for comparison within one controlled series than as absolute numbers. Neither this nor other current procedures neatly separate sulfatides quantitatively from nonsulfatide fractions. This factor was minimized by 1) the significant number and selected variety of simultaneously analyzed samples, 2) recording corrections made for phosphorus content, and 3) the fact that metachromatic lipids which enter the non-sulfatide fraction do so to a greater degree in ML cases where they are clearly increased in the lipid extract to begin with. Consistent biochemical, microscopic, spot test, and gross visual correlations among brains, kidneys, normals, mildly abnormals, and metachromatic cases, reproduced even with different extraction mixtures, support the coherence of the data.

Summary. 1) Metachromatic sulfuric acid esters (SAE) were measured in sulfatide fractions extracted from normal and abnormal white matter and kidney. 2) Slightly lower than average SAE values for white matter were found in early childhood, in old age, and in diseases interfering with myelin metabolism. 3) The average SAE value in normal mature white matter was 58 mg/g. This was 10 times the value for normal kidney and corresponds with the much greater lipid metachromasia in white matter histologically. 4) Statistically significant elevations in sulfatide values were found in white matter and kidney in one disease (metachromatic leucoencephalopathy). 5) This accumulation may be related to an error in sulfatide metabolism, and appears, descriptively, to constitute a "sulfatide lipidosis."

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1. Wislocki, G. B., Singer, M., *J. Comp. Neur.*, 1950, v92, 71.
2. Austin, J. H., *Neurology*, 1957, v7, 716.
3. Lees, M., *Fed. Proc.*, 1956, v15, 973.
4. Folch, J., Lees, M., Sloane-Stanley, G., *J. Biol. Chem.*, 1957, v221, 497.
5. Thannhauser, S., Fellig, J., Schmidt, G., *ibid.*, 1955, v215, 211.
6. Austin, J. H., *Neurol.*, 1957, v7, 415.
7. ———, *Tr. Am. Neurol. Soc.*, June, 1958.
8. Whitelock, O., Ed., *Ann. New Acad. Sci.*, 1957, v69.
9. Gomori, G., *J. Lab. and Clin. Med.*, 1942, v27, 955.
10. Rodnight, R., *J. Neurochem.*, 1957, v1, 207.
11. Grenell, R., May, L., *ibid.*, 1958, v2, 138.
12. McIlwain, H., *Biochemistry and the central nervous system.* Little Brown & Co., Boston, 1955.
13. Cumings, J. Ed., *Cerebral Lipidoses.* Blackwell, Oxford, 1957.

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In vivo Measurement of Radiophosphorus and Radiostrontium Absorption in Rats.* (24628)

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Hamilton(1) demonstrated *in vivo* the absorption of orally administered radioisotopes of Cl, Br, I, Na and K in humans. In the following experiments, this method has been modified to measure absorption of radiophosphorus and radiostrontium in normal adult rats. The isotopes are given by stomach tube, and the radioactivity which accumulates in the

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tail is measured continuously. The method avoids surgical trauma and makes it possible to measure amount and rate of absorption in individual animals under almost normal physiological conditions.

Method. 20 adult female rats of the Sprague-Dawley strain weighing 200-220 g were used. They were lightly anesthetized with urethane-barbitone, or were restrained by wrapping in a towel according to the technic described by Griffith and Farris(2). No significant difference could be attributed to use of anesthesia. The tail was inserted into a special lucite or lead holder which in turn was placed in a standard position under the counter. A thin end window Geiger-Mueller tube was used for P^{32} and Sr^{89}, and a scintillation counter for Sr^{85}. The radioactiivty in the tail was measured continuously with a counting rate meter connected to an Esterline-Angus recording milliammeter. The average diameter of the tail under the counter tube was 8.5 mm, so that a considerable part of the beta radiation from P^{32} and Sr^{89} in the superficial tissue could be detected(3), and practically all of the gamma radiation from Sr^{85}. A preliminary experiment was carried out to determine the validity of using tail radioactivity as a measure of the radioisotope in the whole body. Ten microcuries of the carrier free isotopes P^{32}, Sr^{89} and Sr^{85} were administered by stomach tube in 1 ml distilled water. The rats were killed at various time intervals up to 7 hours after a final *in vivo* measurement of the radioactivity in the tail. The intestinal tract was removed and ashed along with the feces, while the remainder of the carcass was ashed separately. Initially, the intestinal wall was washed, and radioactivity of the gut contents was determined separately, but since the radioisotope of the gut wall did not exceed 0.7% of the administered dose, it was felt that this additional step was not necessary. The quantity of radioisotope present in the carcass after removal of the intestinal tract was taken as net absorption. During *in vivo* absorption measurements tail radioactivity was measured continuously from time of administration of the isotope until the rats were killed, at the end of 3 hours in the case of P^{32}, and 6 hours in the case of Sr^{89} and Sr^{85}. They were then treated as described above, and net absorption of radioisotope was determined. From the ratio of absorbed body radioactivity (by analysis) to radioactivity actually measured *in vivo* in the tail, absorption was estimated at various times during the experimental period. Since Sr^{89} emits only beta radiation, while Sr^{85} emits essentially only gamma radiation, it was possible to make 2 distinct measurements of radiostrontium absorption in the same animal using these isotopes. The initial measurement was made with Sr^{89} as described above, and the measurement then repeated using Sr^{85} and a gamma sensitive scintillation detector with an aluminum filter of 300 mg/cm^2 to eliminate all but gamma rays. A similar procedure was used to distinguish the 2 isotopes in the ashed carcass and intestinal tract.

Results. Ratios of total radioactivity in the carcass to radioactivity measured *in vivo* in the tail have been measured. These ratios were relatively constant up to 3 hours in the case of P^{32} and 5 hours in the case of Sr^{89} and Sr^{85}, but beyond these times, the ratios become erratic. Although the relationship is empirical, it is significant that constant ratios were obtained with the beta emitting isotopes P^{32} and Sr^{89} as well as Sr^{85} which emits gamma radiation and thus suffers very little mass absorption by the tissues The ratios therefore appear to be consistent over the period of active absorption studied in these experiments. The constancy of the ratios indicate that *in vivo* determination of radioactivity in the tail gave a measure of absorption of P^{32} and Sr^{89} during the first few hours after oral administration.

Average values for absorption of P^{32} and Sr^{89} have been plotted against time in Fig. 1. Absorption of phosphate from the gut occurred very rapidly, reaching a peak within the first half hour, and very little additional absorption occurred after 2 hours. The values for 24 and 48 hours represent actual retention of the isotopes in similar groups of animals killed at these times following oral administration. In the case of radiostrontium, there was a latent period of 20-30 minutes in most animals, absorption was slower and continued for a longer time. Since the slope of the curve

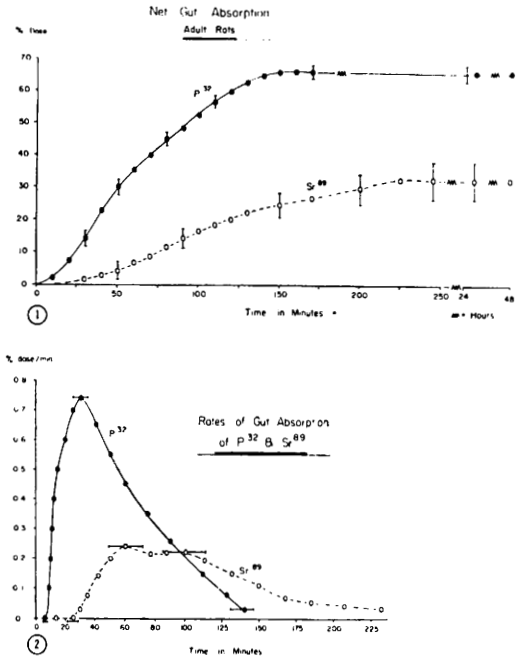

FIG. 1. Net absorption of P^{32} and Sr^{89} in rats. Each curve is avg of 8 individual graphs. Stand. errors are shown.

FIG. 2. Rates of intestinal absorption of P^{32} and Sr^{89} derived from tangents of absorption curve. Stand. errors are indicated.

represents rate of absorption, it is possible to estimate graphically, by drawing tangents, absorption rate in individual animals at various times after administration of the isotope. These averaged values have been plotted in Fig. 2, and are expressed as per cent of the administered dose absorbed per minute.

Since some studies had required the use of anesthetic, a test was made to determine its effect. Seven anesthetized rats absorbed 32.7 $\pm$ 1.4% of oral dose, while 7 unanesthetized rats absorbed 31.4 $\pm$ 3.4% (mean $\pm$ std. error). Thus no essential difference was found. When radiostrontium absorption was measured in the same animal, first with Sr^{89} and then with Sr^{85}, some variation was observed; however in a series of 5 animals, the average absorption on the first occasion (Sr^{89}) did not differ significantly from the average for the second absorption (Sr^{85}).

Discussion. The principal advantages of this *in vivo* method are simplicity, lack of serious physiological disturbance of the animal, and more precise visualization of absorption in individual animals. The characteristic sigmoid curve obtained was similar to that observed in humans by Hamilton(1) using other ions. The difference in the rate maxima in absorption of phosphate and strontium may be due to differences in the site and gut transfer rate of these ions in different parts of the intestinal tract. Weissberger *et al.*(4) observed in the dog that P^{32} appeared in the blood within 5 minutes after oral intake, and reached a maximum at 30 minutes. This suggests active absorption high in the intestinal tract and supports the observations reported here. On the other hand, there was a delay of 20-30 minutes in most rats before any significant amounts of radiostrontium were absorbed, and the whole absorption process was slower. This delay may have practical significance in the case of accidental ingestion of radiostrontium, since it indicates a period during which prompt treatment might be effective. However, most of the absorption of radiostrontium which did take place occurred within the first 5-6 hours, as was observed by Jones and Coid(5). The use of this short period allowed more direct measurement of absorption by reducing endogenous Sr excretion to 2.1% of oral dose.

The double tracer technic, using Sr^{89} and Sr^{85}, provides a means of comparing strontium absorption in the same animal under normal and then under experimental conditions. It should prove useful for studying treatments designed to reduce radiostrontium absorption, or for investigating factors which may modify it.

Summary. 1. An *in vivo* method has been described for studying radiophosphorus and radiostrontium absorption from the intestinal tract in rats. The method is simple, avoids surgical trauma and permits visualization of the absorption process in individual animals. Rate of absorption at various times may also be estimated. This is not possible with the standard balance studies. Anesthesia is not necessary, though no difference of Sr^{89} absorption was found when anesthesia was used. 2. Absorption of radiophosphorus was very rapid, reaching a peak at 30 minutes. In most animals, there was a delay of 20-30 minutes be-

fore significant amounts of radiostrontium were absorbed, and the whole process was slower. 3. It was possible to measure radiostrontium absorption on two different occasions in the same animal, first with Sr^{89} and then with Sr^{85}. This should prove of value in studying factors affecting strontium absorption.

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1. Hamilton, J. G., *Am. J. Physiol.*, 1938, v124, 667.

2. Farris, E. J., Griffith, J. Q., Editors. *The Rat in Laboratory Investigation.* J. B. Lippincott Co., Philadelphia, 1942, 21.

3. Baglioni, T., Garavaglia, C., Perussia, A., Polvani, C., Silvestroni, B., *Biol. Lat.*, 1955, v8, 571.

4. Weissberger, L. H., Nasset, E. S., *Am. J. Physiol.*, 1943, v138, 149.

5. Jones, H. G., Coid, C. R., *Clin. Sci.*, 1956, v15, 541.

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Evaluation of Laboratory Diagnostic Procedures with A/Asian Influenza. (24629)

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The recent influenza epidemic stimulated many virus diagnostic laboratories to determine whether recommended laboratory procedures were adequate and to evaluate alternative diagnostic procedures(1). Throat washings and serum specimens we received were processed by standard procedures, and results analyzed to investigate the following: 1) comparison of complement fixation and hemagglutination-inhibition tests for routine diagnosis, 2) relationship between time of appearance of antibodies titrated by above methods, 3) correlation between increased antibody titers and virus isolation, and 4) comparison of several isolation methods.

Materials and methods. The majority of acute and convalescent serums were obtained from individuals 10 to 65 years of age. Samples were submitted from many areas of the United States. Selection of patients and collection of throat washings were made by numerous physicians and health officers with varying degrees of experience. Due to this fact, specimens were often subjected to suboptimal conditions for storage and transportation. Infected allantoic fluids served as specific antigens in both serologic tests. These were produced by injection of approximately 10,000 EID_{50} by the allantoic route in 11-day embryonate eggs. Following 48 hours incubation at 37°C, the eggs were chilled several hours, fluids harvested, clarified by low speed centrifugation and stored at 4°C. Virus strains used were A/Asian/Japan/305/57, A/Denver/1/57, and B/GL/1739/54. *Serologic procedures.* Complement fixation (CF) test employed was the standard technic in the Virus and Rickettsia Section. With this method, 0.1 ml of serum and antigen dilutions are mixed with 0.2 ml of complement and later sensitized sheep erythrocytes to give a final total volume of 0.6 ml. Optimal dilutions of antigen as determined in box-type titrations were used. Complement titers were measured in presence of each antigen, and dilutions contained 2 optimal units. The test was incubated overnight at 4°C followed by 20 minutes at room temperature, at which time sensitized cells were added. The test was read after incubation at 37°C for one-half hour. The tube containing greatest dilution of serum in which less than 50% hemolysis (estimated) occurred was considered as the positive end point. Hemagglutination-inhibition (HI) tests were performed as previously described

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