That gamma amounts of protein produce unresponsiveness in adult guinea pigs contrasts sharply with the massive doses of proteins needed for tolerance induction in adult rabbits(10). Should the mesenteric vein route for administration of minute amounts of antigens be successful in species other than the guinea pig and, perhaps, in inducing tolerance of homografts (experiments in progress), the necessary foundation will be provided to structure a single mechanism for the phenomenon of tolerance.

Summary. Delayed hypersensitivity and circulating antibody production to a hapten (picryl chloride) and to a protein antigen (bovine gamma globulin) were regularly suppressed in mature guinea pigs injected twice into mesenteric veins prior to sensitization, with hapten or protein in gamma amounts.

ADDENDUM: Following preparation of this manuscript, a report by D. W. Dresser has appeared (*Immunol.*, 1962, v5, 378) on induction of unresponsiveness in adult mice by parenteral administration of minute doses of a protein antigen.

The authors wish to thank Dr. E. J. Hehre for help with this manuscript and Miss Rita Hixon for skilled technical assistance.

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Received July 9, 1962. P.S.E.B.M., 1962, v111.

Measurement of Total Body Iron[59] in Animals Using Whole-Body Liquid Scintillation Detectors. (27718)

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The construction of large-volume, 4-pi liquid scintillation detectors permits direct measurement of total-body gamma radiation in humans and animals(1-3). The purpose of this paper is to describe the reliability of this method in determining the retention and rate of excretion of Fe^{59} in rats. The biologic problems encountered with iron are described.

Materials and methods. Adult male Canadian Hooded rats weighing 235-411 g were used in the study. Rats were raised in a pathogen-free environment and housed in wire cages. Their diet contained 15 mg of iron per 100 g of dry weight and average daily ingestion was estimated to be 1.5-2.5 mg of iron. Some rats were iron depleted by bleeding 4 ml weekly for 4 weeks prior to study and fed a milk-powder iron-deficient diet. Other rats were iron-loaded with 4 intramuscular injections of 25 mg of elemental iron as dextran-iron at weekly intervals.

The test dose of iron consisted of 0.1 or 0.25 μc of Fe^{59} as citrate in 0.25 ml of water with various carrier doses of elemental iron as ferrous sulfate. Oral test doses were administered through an olive-tipped 17-gauge endo-

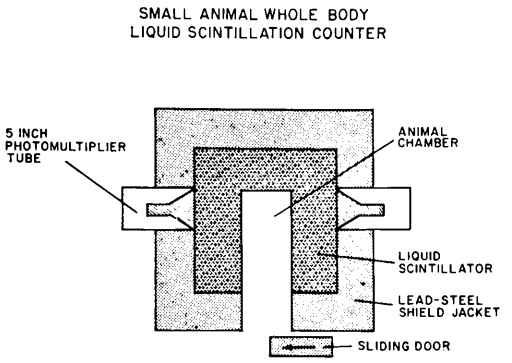

FIG. 1. Specimens containing gamma-emitting nuclides were placed in the internal cylinder. Scintillation solution surrounded this chamber except at one end of tank. Gamma emissions were converted into light impulses in scintillator liquid. These signals were detected by two 5-inch photomultiplier tubes. Amplification and discrimination of these impulses permitted efficient and selective quantitation of radionuclides. Radiation from external sources was decreased by the 3-inch lead-steel shield surrounding liquid scintillator.

esophageal tube after a 16-hour fast. Parenteral doses were administered by intravenous injection into the tail vein.

Body radioactivity (0.6 Mev- ∞) was measured in a small animal whole-body liquid scintillation counter. This was a 4-pi counting system similar to that described by Woodward(2), fitted with two 5-inch laterally disposed photomultiplier tubes (Fig. 1). Calibration of the counter for Fe^{59} required determination of absolute efficiencies for standards ranging in weight from 10 to 750 g. Radioactivity was detected less efficiently in a large mass because of self-absorption. A log-log plot of data demonstrated a linear relationship between sample weight and detector

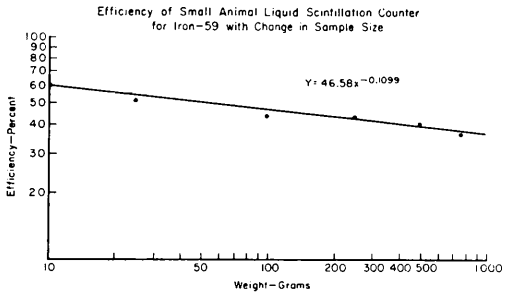

FIG. 2. Efficiency of detector decreased with increasing weight of sample. Calibration was performed with 250-ml water-filled plastic bottles containing 0.25 μc of Fe^{59}.

efficiency (Fig. 2). Thus in the animals studied (235-411 g) counting efficiency varied less than 2%. The relation between count rate and dose of Fe^{59} was almost linear. Efficiency declined from 44.6 to 42.1% with increase in net count rate from 165 to 4000 counts per second. Error was less than 1% when measurements were corrected for resolving time loss (Fig. 3)(4).

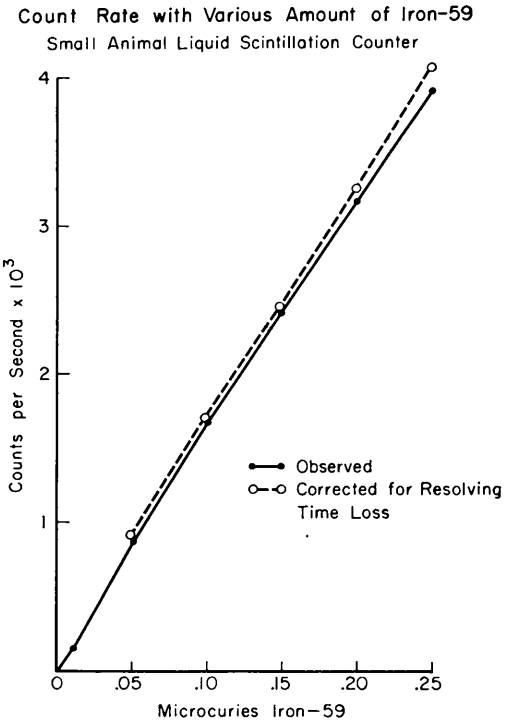

FIG. 3. Relationship between Fe^{59} dose and count rate was almost linear.

Rats were placed in vented ¾-quart ice cream cartons for counting. Gross count was determined from two 100-second counting periods. Prior to administration of Fe^{59} each animal's radioactivity was assayed in the counter. Previously undosed rats had insignificant counts. Net count after dosing was calculated by subtracting measured background radioactivity and that due to any previously administered radioisotope.

Standards were prepared for each experiment by measuring a test dose of Fe^{59} into 250-ml water-filled plastic bottles. The appropriate standard was counted for 200 seconds each time Fe^{59} retention was measured

in the rats. The ratio of animal count 2 hours after the test dose (r_0), to that of the reference standard (s_0) was used as the 100% reference value. Similar proportions (r_x/s_x) at later intervals were compared to the reference value to determine the proportion of retained radionuclide.

$$\frac{r_x/s_x}{r_0/s_0} \times 100 = \% \text{ retention of radionuclide}$$

at time x. This comparison of the activity of specimens with a reference standard at each determination obviated the need for correction for physical decay of the radionuclide and fluctuations in counter efficiency.

Stool collections were placed in 250-ml plastic bottles filled with distilled water. Radioactivity was determined as in the test animals.

Results. Exp. 1. Comparison of test dose and reference standard. This study was performed to show the relation of test dose and standard and reproducibility of the dosing procedure. Thirty rats received an oral dose of 0.10 μc of Fe^{59}. Counts made 2-3 hours after dosing were compared and the ratio r_0/s_0 was 0.9883 with a standard deviation of 0.0141. Maximum variation was 0.9338 to 1.1142. Close correlation between count rate of reference standard and dosed rats demonstrated the reproducibility of dose measurement, delivery to the animal and retention by rats during the period of maximum absorption from the gut.

Exp. 2. Retention of a parenteral dose of Fe^{59}. Retention of a parenteral dose of Fe^{59} (0.25 μc) was measured twice weekly in 4 normal rats. Cumulative stool collections were assayed weekly for Fe^{59}. Each rat lost 5-7% of the test dose during the week following injection. Then there was gradual loss of radioactivity in stool at a daily rate of 0.2% of the test dose. After 150 days observation the measured sum of Fe^{59} retained by rats plus that lost in feces was 96% of the test dose (Fig. 4). The reliability of the counter was shown by periodic assay of Fe^{59} in rats and their excreta over a period of observation of more than 3 half-lives of the isotope. The biphasic loss of iron in the stool of individual rats extending through the period of observation was similar to that ob-

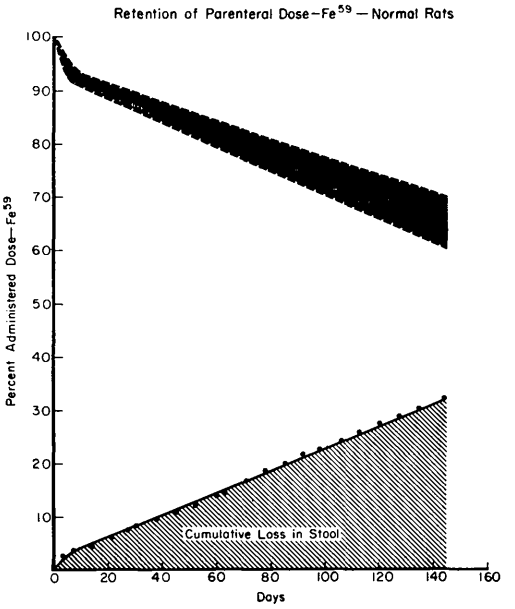

FIG. 4. Body retention of a parenteral dose of Fe^{59} was assayed periodically in 4 normal rats. Avg retention (points in upper slope) was plotted weekly. Range of retention by group was within shaded area. Fe^{59} lost from rats was recovered in fecal collections (lower curve represents cumulative loss per rat).

served by Chappelle in a colony of mice(5).

Exp. 3. A controlled test situation for absorption studies. A. Carrier iron. The importance of the carrier dose of iron in establishing a test dose was studied. Eighteen normal rats weighing 235-355 g were divided into 6 equal groups and given doses of carrier iron varying from 0.05 to 2.0 mg with 0.25 μc of Fe^{59}. Mean absorption varied from 3.7 to 27% of the test dose and was inversely proportional to the size of the carrier. The range of individual values for each dose also decreased with increasing amounts of carrier iron (Fig. 5). A linear relationship existed between the percentgae of Fe^{59} absorbed by rats and the carrier dose when the latter was expressed logarithmically.

B. Period of fast. The period of fast prior to the test dose was studied in 30 rats weighing 250-300 g. Each rat received 0.25 μc of Fe^{59} with 0.5 mg of carrier iron after varying periods of fast. A gradual increase of absorption of iron was observed as the period of fast was lengthened to 48 hours. However, pro-

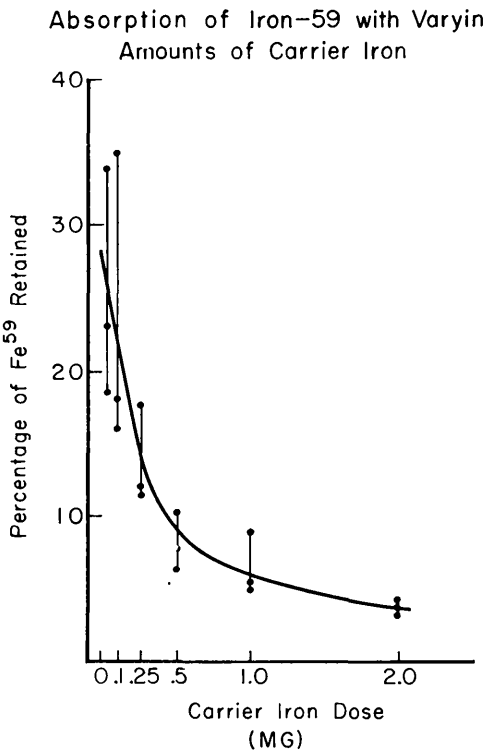

FIG. 5. Increased amounts of carrier iron decreased absorption of Fe^{59} in test dose.

longed fasting decreased the absorption of Fe^{59} (Table I).

C. Weight of animal. The correlation between weight and iron absorption by test animals was studied in 22 rats. The animals weighed 239-411 g and each received 0.25 μc of Fe^{59} with 0.25 mg of carrier iron. Less absorption of the test dose was observed in larger animals (Fig. 6). The product-moment correlation coefficient was –0.604 (p –0.01) indicating a significant inverse relationship.

D. Diet additives. Food additives can be a source of variation in iron absorption studies. The addition of 10 mg of ascorbic acid to a test dose of 0.25 μc of Fe^{59} in a 0.5 mg carrier dose significantly enhanced absorption. Oral dosing of rats with 100 mg of neomycin thrice daily for 4 days prior to giving the same dose decreased absorption (Table II).

TABLE I. Duration of Fast Before Test Dose of Fe^{59}.

None	12 hr	24 hr	48 hr	168 hr
2.1	7.3	8.7	12.8	0.9
4.3	9.0	11.5	15.7	1.1
7.4	12.9	14.2	21.1	1.5
11.7	13.5	17.7	29.8	1.7
16.2	16.8	20.1	33.7	1.9
22.3	18.2	22.4	42.3	2.2
Mean 10.7	13.0	15.8	25.9	1.6

Figures expressed as % of test dose retained.

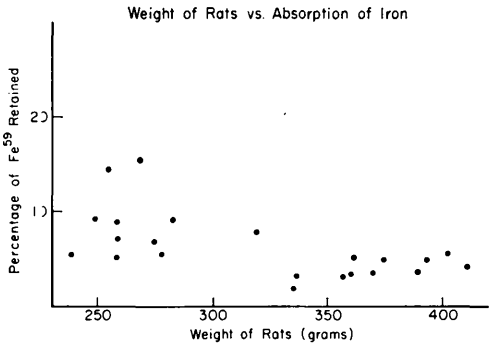

FIG. 6. Larger normal rats absorb less of test dose of Fe^{59}.

E. Individual variability. When the 4 factors above were controlled individual variations in iron absorption were studied in 10 rats weighing 239-281 g. Each animal was given at weekly intervals a dose of 0.10 μc of Fe^{59} in a 0.5 mg carrier dose for 3 doses. Less variation occurred in repeated determinations in single animals than among the individual animals (Table III). Analysis of variance showed a mean square of 19.5 (2 DF) between replicate determinations and 29.4 (9 DF) when rats within single studies were compared.

TABLE II. Effect of Ascorbic Acid and Neomycin Upon Iron Absorption.

	Normal	Ascorbic acid	Normal	Neomycin
	8.5	15.7	7.0	.9
	11.8	17.9	12.7	1.3
	14.1	26.8	14.4	2.6
	16.0	29.6	18.2	2.8
Mean	12.6	22.5	14.1	1.9

Figures expressed as % of test dose retained.

A test situation was established which permitted repeated measurements of iron absorption and in which the animal could be used as its own control.

Exp. 4. Absorption of iron associated with body iron stores. Absorption of a test dose of

TABLE III. Variation in Iron Absorption. Individual variability vs. group variability.

Rat No.	1st dose	2nd dose	3rd dose
1	14.5	6.8	8.5
2	5.1	3.3	2.2
3	9.2	5.9	4.3
4	9.2	10.3	8.6
5	5.5	5.3	3.4
6	7.2	6.9	4.4
7	6.8	3.2	5.3
8	5.5	4.0	4.6
9	8.8	7.3	7.1
10	15.4	14.2	11.9
Mean	8.7	6.7	6.0
S.D.	3.6	3.4	3.0

Figures are % of test dose absorbed.
Carrier 0.5 mg.

iron (0.25 μc Fe^{59} in 0.5 mg carrier) was determined in normal, iron-depleted and iron-loaded rats. Relative equilibrium was achieved within 8 days. Normal rats absorbed 5-18% of the test dose. Iron-depleted animals retained more than 20% and iron-loaded rats less than 3% of the dose (Table IV). Assay of the radioactivity in cumulative stool collections showed over 95% of lost Fe^{59} was recovered. Coprophagia or blood licking added to the absorption of Fe^{59}. Normal, undosed cage-mates in this study absorbed 0.1-0.8% of the radioiron administered to each test animal.

Discussion. Substitution of liquid phosphors for crystal scintillators permits direct assay of γ-emitting nuclides in volumes of unusual size or shape(1,2,6). Similar to crystal scintillators they provide efficient assay of radionuclides and permit differentiation of γ-emitting nuclides if appropriate discriminator circuits are used(7,8).

TABLE IV. Iron Absorption in Rats.

Normal	Iron depleted	Iron loaded
5.4	21.5	.3
6.4	28.2	.5
6.8	31.7	.5
7.5	35.2	.8
8.3	35.8	1.2
10.7	35.9	1.7
14.6	37.6	2.4
17.3	39.8	2.6
Mean 9.62	33.21	1.25

Figures expressed as % of Fe^{59} retained by rat 8 days after test dose.

The development of small-animal whole-body liquid scintillation counters provides a reliable method of measuring radionuclides in live small animals and in specimens from these test animals after dilution to achieve appropriate geometry. The small quantity of radioisotope needed reduces cost and radiation hazard and permits performance of repeated studies. The efficiency and stability of large-volume liquid scintillation detectors allows accurate measurement of retention of radioisotopes during prolonged periods. Obviously, this period is limited by physical decay, biologic life and dose of the radionuclide.

Fe^{59} was used to exemplify the use of this detection system in biologic studies. Reliability of the counter was demonstrated by repeated measurements of body retention and fecal loss of Fe^{59} during 3 half-lives. A test situation was established which permitted accurate determination of absorption and rate of loss of this radionuclide. Control of known variables allowed comparison of repeated absorption studies in the same animal.

Summary. Iron absorption and total body retention was measured in rats using radioiron[59] and a whole-body liquid scintillation counter. Whole-body liquid scintillation counting provides an efficient and reliable method of assaying small quantities of γ-emitting nuclides in live animals and biologic specimens.

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