vasan(10). Our values with normal rats, however, compared favourably with theirs. In their case a decrease in α_1-globulin with significant increase in β-globulin was observed, whereas under our conditions an increase in α_2-globulin with a decrease in γ-globulin was observed.

Summary. Different fractions of serum proteins were determined by paper electrophoresis in folic acid deficient and pair-fed normal rats. Total serum protein was low and plasma fibrinogen was significantly high in the deficient animals. There was a significant decrease in albumin and γ-globulin, considerable increase in α_2-globulin, with no significant change in α_1 and β-globulin fractions in the serum of folic acid deficient rats.

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Exchange of Radioactive Magnesium in the Rat.*† (24584)

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Changes in extracellular concentration of magnesium have been implicated in various pathological states and in mammalian hibernation. The clinical aspects have been reviewed by Flink(1) and Elkinton(2) and the role of magnesium in hibernation by Riedesel(3). The recent availability of a radioactive isotope, Mg^{28}, has provided a tool for investigating the dynamic equilibrium between extracellular magnesium and that in the cells and/or skeleton. The present report concerns the exchange of magnesium between plasma and soft tissues in the rat.

Methods. Mg^{28} is an isotope emitting both gamma and beta radiation with a half-life of 21.3 hours. It decays to Al^{28} which also emits gamma and beta radiation with a half-life of 2.3 minutes, and this daughter decays to stable Si^{28}. The isotope, with Mg^{24} carrier, was obtained from the Brookhaven National Laboratory and was prepared to yield a solution containing 150 meq/l magnesium. Twenty-six male rats ranging in weight from 290 to 325 g were each injected with about 0.5 ml of this solution intraperitoneally. The injections were made from a tuberculin syringe which was weighed on an analytical balance before and after each injec-

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tion. At intervals after injection each rat was anesthetized and exsanguinated through a needle in the abdominal aorta. One ml samples of plasma were taken for radioactivity determination and chemical analysis for magnesium. The red cells were washed twice in ice-cold saline and radioactivity of 1 ml of packed cells was counted. The other tissues sampled included liver, left ventricular myocardium, kidney, brain (whole cerebral hemispheres), erythrocytes, testis, diaphragm, and gastrocnemius. In 14 rats the radioactivities of the erythrocytes were determined but not those of the brain, and vice versa in the other 12. A similar experiment was conducted with 11 immature male rats weighing 150 ± 10 g. The tissues sampled were as above, including brain but excluding erythrocytes. The uptake of radioactive magnesium by the rat diaphragm *in vitro* was also studied. Hemidiaphragms from rats weighing 150 ± 10 g were dissected and incubated for 50 minutes in a solution containing radioactive magnesium. The medium was a Krebs-Ringer, bicarbonate-CO_2 buffered solution containing 100 mg % glucose and 200 units I insulin. Each hemidiaphragm was weighed and placed in a separate 25 ml Erlenmeyer flask with 3 ml of the nutrient solution and shaken at 180 min. in a water bath at 37°. They were removed at intervals and their radioactivities measured. Incubation time of each hemidiaphragm was different from that of the other hemidiaphragm from the same animal. Finally, uptake of radioactive magnesium by stimulated muscles was compared with that of unstimulated muscles. Two male rats weighing about 300 g were anesthetized with sodium pentobarbital and the sciatic nerve exposed in each thigh. A stimulator electrode was placed under each nerve but only one side was actually stimulated. The stimulations were from a Harvard stimulator at 2 per second. The rats were killed after 27 and 52 minutes, respectively, and radioactivities of the gastrocnemii from the 2 sides were compared. Plasma and tissue samples were placed in a well-type scintillation counter and counted for 2 minutes or until 2000 counts had been recorded. After suitable corrections for decay and the injected dose per kg body weight, specific activities of plasma and tissue magnesium were calculated. The specific activity of the magnesium in each tissue was divided by that of the plasma magnesium at time of sampling. This gives "relative specific activity" (r.s.a.), which indicates the degree of equilibration of the magnesium in plasma and tissue. A value of 1 indicates complete equilibration. The plasma samples were analyzed for magnesium by the method of Orange and Rhein(4). This is essentially a colorimetric determination of a magnesium-Titan Yellow complex in the presence of a colloidal material, in this case polyvinyl alcohol. For the other tissues mean values from the literature for the absolute magnesium content were used(5). Calculations were made using values for each extreme of the range reported in the literature and where no such range was given, it was arbitrarily assumed to be ± 25%. In Fig. 1, b, c, d, and f, the solid line shows increase of r. s.a., using the mean value for magnesium content of the tissue and the broken lines show the 2 ranges.

Results. A. *Plasma.* The decay of the standardized specific activity of plasma magnesium in mature rats is shown in Fig. 1 a. There are probably 2 exponential processes. The slope of the slower component was fitted by eye and extrapolated to the y-axis so that value of the slow component could be determined at each experimental interval. These values were subtracted from the experimentally determined activities and the differences plotted semilogarithmically. The slope of this new line, plotted as the broken line in Fig. 1 a, is taken as that of the faster process. It was found to be –0.87, that of the slower component, –0.04. In the immature rats the slopes were –0.93 and –0.07, respectively.

B. *Tissues.* In the exchange of plasma magnesium with that in the cells, tissues seem to fall into 2 classes. In liver, heart, and kidney the exchange is rapid and reaches complete equilibrium in about 3 hours. In brain, testis, red cells and skeletal muscle the exchange is not complete after 7 hours. *Liver, heart, and kidney.* The increase of relative standardized specific activity (r.s.a.), of heart

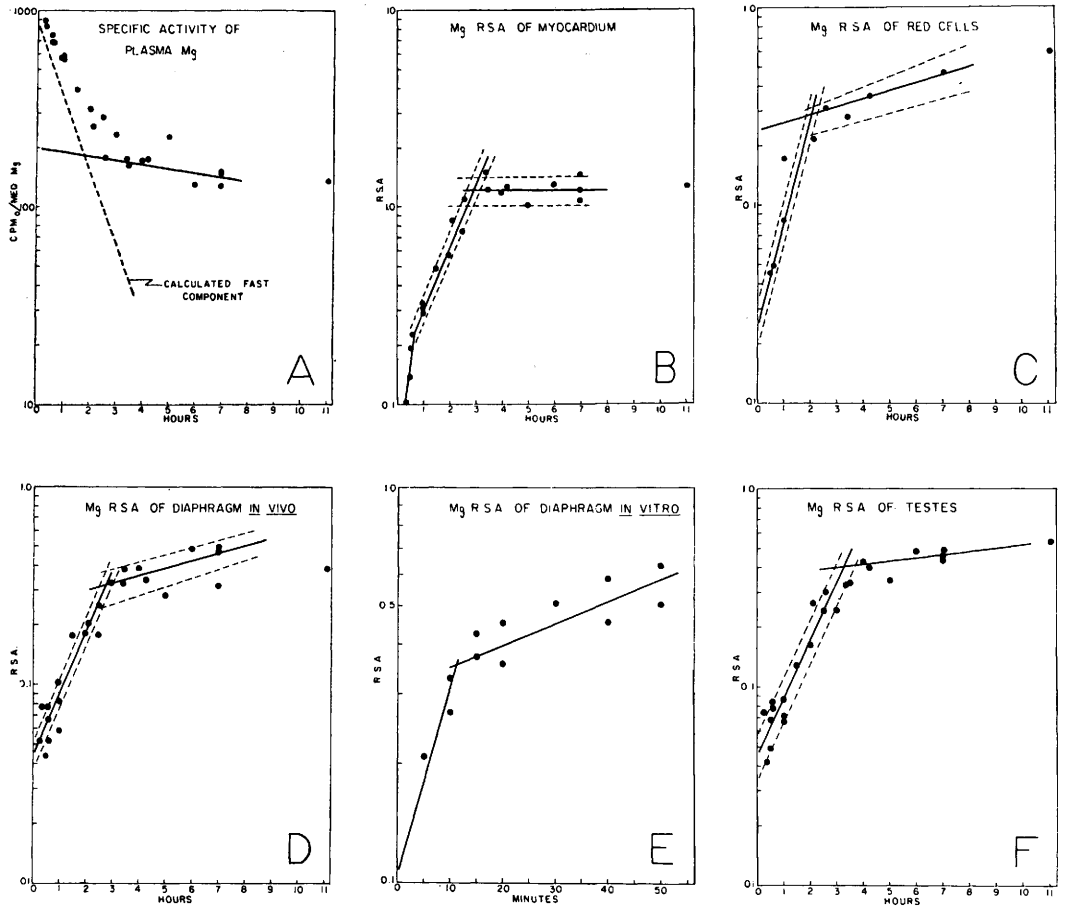

FIG. 1. R.S.A. is relative specific activity. R.S.A. $= \frac{\text{specific activity of tissue Mg}}{\text{specific activity of plasma Mg}}$. Broken lines indicate range of R.S.A. calculated from normal range of Mg content in the appropriate tissue.

muscle in mature rats is shown in Fig. 1 b. The graphs for liver and kidney are very similar to this. In each case there appear to be 2 fast components with an inflection at about one-half hour after injection. Complete equilibration is achieved at about 3 hours. Overall increase before equilibration is reached has a slope of 0.78 which is similar (but with the reverse sign) to that for plasma decay. The comparable value for heart in the immature animals is 0.74. *Red cells.* The exchange of magnesium in red cells is shown in Fig. 1 c. The data do not permit an analysis of the components of the exchange but it is clear that there is some magnesium in rapid exchange with that in the plasma and at least one other magnesium "compartment" in relatively slow exchange. The exchange is only 45% complete after 7 hours and in a single rat sacrificed after 11 hours equilibration was 60% complete.

Skeletal muscle. The increase in the r.s.a. in diaphragm is shown in Fig. 1 d. The scatter of points is greater than in some of the other tissues but an inflection at about 3 hours is apparent. In the gastrocnemius r.s.a. is lower but follows a similar pattern. After 7 hours equilibration is 50% complete in the diaphragm and 20% in the gastrocnemius. The slope of the initial increase in the r.s.a. in diaphragm is 0.75.

Diaphragm in vitro. The initial process in equilibration of the diaphragm *in vivo* is strikingly similar to that of the rat diaphragm

TABLE I. Uptake of Radioactive Magnesium by Stimulated Gastrocnemius.

Duration of stimulation, min.	cpm/kg × 10^{-3}	
	Stimulated	Unstimulated
27	106	44
52	271	169

incubated *in vitro*, shown in Fig. 1 e with a different time scale. Slope of the *in vitro* equilibration is 0.78. The rapid increment of the r.s.a. during the first 10 minutes of incubation represents presumably the exchange of magnesium in the interstitial fluid.

Stimulated gastrocnemius. Table I lists the results of *in vivo* stimulation of the gastrocnemius. In both cases the stimulated muscle has much higher radioactivity than the unstimulated.

Brain and testes. The increase in the r.s.a. of the magnesium in testes is plotted in Fig. 1 f. Equilibration is only 45% complete after 7 hours and a single value at 11 hours shows 55% equilibration. Here again there are 2 components analagous to those in the plasma. The pattern of exchange in the brain is similar but the initial increase is slower and equilibration after 7 hours is 25%. In the immature rats the slope of initial increase in r.s.a. of testes is the same as in the older animals but is continued for longer so that after 7 hours the testes are 70% equilibrated rather than 50%.

Discussion. The use of mean values for magnesium content of the tissues is evidently adequate for interpretation of the radioactivity data. In each case (Figs. 1 b, c, d, and f), the results calculated from the extremes of each range of values are not significantly different from the mean and do not affect the estimate of the time at which equilibration is reached. This is true even of those tissues in which the range is assumed to be as high as ± 25%. Furthermore, measurement of the slope is independent of absolute magnesium content of the tissue.

Following injection of the magnesium isotope, the tissues can become radioactive either by net uptake of magnesium or by exchange of intracellular with extracellular magnesium. The former possibility must be considered. The isotope is not available free of carrier, and so to achieve sufficient radioactivity for detection in the latter phases of the experiment it is necessary to inject more magnesium than is ideal in a tracer experiment. The animals are thus subjected to a slight magnesium load. The very rapid increment of radioactivity of liver, kidney, and heart during the first 30 minutes may be interpreted as a deposition of some of the injected load. As these organs comprise 15% of the body weight and the total load to be distributed amounts to only 0.064 meq, the increase would lie within the normal range of variation in chemical composition even if none were deposited elsewhere.

The most striking aspect of these results is that the organs clearly fall into 2 classes with respect to their uptake of radioactive magnesium. The organs in which the magnesium exchanges rapidly and completely with that of the plasma are the liver, heart, and kidneys. Noonan *et al.*(6) found that in this same group of organs the potassium was in rapid exchange with radioactive potassium in the plasma and that the potassium of brain, testes, and erythrocytes was slow in equilibrating. They also demonstrated, however, that skeletal muscle is intermediate between the 2 groups, and in this respect the results with magnesium are different. Equilibration of muscle magnesium is just as slow as that in the red cells for example, suggesting that the magnesium in muscle is more tightly bound than is the potassium. In skeletal muscle (as in all the "slow" organs) about 20% of the magnesium can exchange readily with the extracellular fluid at a rate with a half-time similar to the main process in the plasma decay. The remainder of the magnesium is relatively inexchangeable.

Within each of the 2 groups of organs there is remarkable similarity in equilibration rates of such disparate tissues as diaphragm and testes, for example. This could be accounted for either by a similarity in permeability of the membranes to magnesium or by the existence of 2 (or more) states of magnesium in the cell, each with its characteristic exchange rate. The latter proposal is more consistent with the observations that there are 2 processes in equilibration in the "slow" organs and that the faster of these processes has the same

rate as the single process in the "fast" organs. On the one hand we may consider a "free" magnesium with a turnover time of 1.2 hours and on the other hand, a "bound" magnesium with a turnover time of 25 hours.

In liver and kidney, all of the magnesium would be in the rapidly exchanging state and in skeletal muscle more than half the magnesium would be in the slowly exchanging state. The intimate association of magnesium with the contractile elements of muscle is also consistent with this proposal and it is especially noteworthy that a *stimulated* skeletal muscle shows a more rapid exchange than an unstimulated muscle. The possible storage of magnesium load in the liver, etc., is of interest but by analogy with other electrolytes the skeleton presumably contains the principal reserve of magnesium.

The decrease of specific activity in the plasma of immature rats is slightly faster than in the older rats. This is to be expected even if only from a consideration of their shorter circulation time. In the testes of immature rats initial rate of increase of r.s.a. is the same as in the older animals but it is maintained for longer so that after 7 hours the testes are 70% equilibrated rather than 50%. The implication is that the less mature testes contain more of the rapidly exchangeable magnesium. Otherwise there are no striking differences between mature and immature rats.

Summary. Mature and immature rats were injected with radioacitve magnesium and distribution of the isotope in various tissues was measured. The exchange with plasma magnesium was rapid and complete within 3 hours in liver, kidney, and heart muscle. In brain, testes, erythrocytes and skeletal muscle there was a rapidly exchanging component similar to that in liver, etc., but more than half the magnesium exchanged very slowly. The similarity between functionally disparate tissues in each of the 2 groups suggests that the magnesium is present in 2 or more physiologic states. In one state the turnover time is 1.2 hours and in the other it is 25 hours. The exchange was faster in muscles which were stimulated to contract repeatedly than in resting muscles. In immature rats uptake of radioactive magnesium by the testes was slightly faster than in the mature animals and net decay of plasma activity was faster but there were no other significant differences.

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Effect of Salicylates on Respiration and Phosphorylation in Heart Mitochondria. (24585)

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The observations that salicylate and dinitrophenol significantly increase basal metabolic rate in intact man(1-3) and animal(4-5) have been attributed to the ability of these phenols to uncouple oxidative phosphorylation(6-7). In view of the efficacy of salicylates in treatment of rheumatic fever and rheumatoid arthritis, the cardiovascular effects of salicylate-induced hypermetabolism have received some attention. Tenny and Miller(5) demonstrated in the dog that major cardiovascular adjustments to salicylates

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