

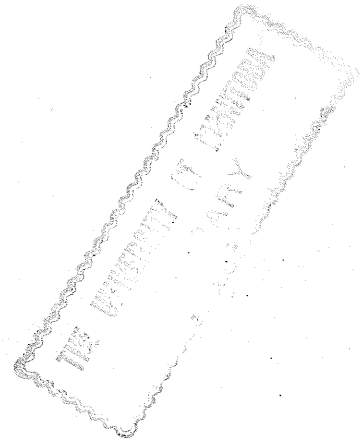
A STUDY OF PLASMA MAGNESIUM IN
DISORDERS OF THE THYROID GLAND WITH A REVIEW
OF THE METABOLISM OF MAGNESIUM

A Thesis
Presented to
the University of Manitoba

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Master of Science

by
James Bert Cosgrove, M.D.

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CHAPTER I

INTRODUCTION

A. RELATIONSHIP OF PLASMA MAGNESIUM TO THYROID FUNCTION

1. Direct evidence. In recent years a difference of opinion has developed regarding the amounts of non-diffusible magnesium in the plasma of patients with various disorders of the thyroid gland. It has been claimed that this non-diffusible magnesium is increased in hyperthyroidism and reduced in myxedema (Soffer et al. 1939, 1941, Dine and Lavietes 1942) but other investigators have obtained contrary results (Cope and Wolff 1942, Bissell 1945). Because of the conflicting evidence the alterations of non-diffusible magnesium in plasma associated with disturbances of thyroid function cannot be regarded as established.

2. Indirect evidence. The possibility that the metabolism of magnesium may be associated with the function of the thyroid gland, is indicated by other evidence. Firstly, in experimental animals, a low serum magnesium produced by magnesium deficiency is associated with vasomotor instability and hyperirritability of the nervous system (Greenberg and Tufts 1938). Both of these are common in hyperthyroidism and it is conceivable that they may be due to the fall in diffusible magnesium, and so of ionizable magnesium, which

Soffer et al. (1939) reported to occur when the non-diffusible portion was raised. Secondly, the regression of symptoms of Grave's disease following injections of magnesium has been reported (Heubner 1939). Thirdly, the thyroid gland is known to exert a marked influence on calcium metabolism, and in hyperthyroidism there is an increased excretion of calcium which may result in general decalcification of the skeleton. The interactions of magnesium with the metabolism of calcium are beginning to be recognized, particularly with respect to the interrelationships of magnesium, phosphatase, and calcium in the processes of bone formation. Therefore, the disorder of thyroid function which results in hyperthyroidism and causes a disturbance of calcium metabolism might be expected to affect the metabolism of magnesium also. Fourthly, Suomalainen (1939) has demonstrated that serum magnesium is increased markedly in hibernating animals. He has shown also that the concentration of magnesium in the serum of sleeping human subjects is raised. In both of these conditions it is recognized that basal metabolism is lowered. In view of the findings of Soffer et al. (1941) that the percentage of diffusible serum magnesium was increased by 100 per cent in myxedema it seems possible that some association may exist between thyroid activity and the metabolism of magnesium.

B. REASON FOR STUDY AND STATEMENT OF PROBLEM.

1. Reason for study. Although the indirect evidence suggests

some association between the metabolism of magnesium and the function of the thyroid gland, the direct evidence cannot be assessed because of the conflicting results which have been reported. It is clear that further data are required before a definite conclusion can be made. The confirmation of an association between the metabolism of magnesium and thyroid function would add to the knowledge of thyroid physiology and might lead ultimately to new methods of diagnosis in diseases of the thyroid gland.

2. Statement of problem. The problem of this investigation was to determine the plasma levels of magnesium in a variety of subjects, particularly those with disorders of the thyroid gland, in order to ascertain if the function of the thyroid gland was intimately associated with the metabolism of magnesium.

CHAPTER II

REVIEW OF THE LITERATURE

A. INTRODUCTION

1. Scope of the review. It was considered that an investigation of the alterations in plasma magnesium in disorders of thyroid function required a knowledge of the occurrence and distribution of magnesium in the animal organism and an understanding of its function in the body. Accordingly, an attempt was made to review the literature pertaining to these topics from 1900 to the present.

2. Sources of information. References were sought in the Quarterly Cumulative Index Medicus under the heading "Magnesium". However, it was found that some references were not included under this caption because much of the experimental data regarding the biological role of magnesium has been derived incidentally to studies of calcium metabolism. Therefore, a search was made also under the titles Calcium, Blood, Urine, Bone, Muscle and Thyroid. In spite of this, it was discovered that a few pertinent articles were not included in the Quarterly Cumulative Index Medicus. Furthermore, many of the journals prior to 1916 were not available. Hence, use was made of Physiological Reviews, Annual Reviews of Biochemistry and text books. Excellent bibliographies concerning the literature prior to 1939 are presented by Schmidt and Greenberg (1935) and Duckworth (1939). An effort was made to review as

completely as possible all the English literature concerning this subject which has been recorded from 1939 to January 1948. In this respect the Quarterly Cumulative Index Medicus was supplemented by the Annual Reviews of Biochemistry, The British Chemical Abstracts and recent text books.

B. METHODS OF ESTIMATING MAGNESIUM

1. Methods used. Four major methods have been used for the determination of magnesium in animal tissues; the gravimetric, molybdenum blue, titan yellow and hydroxyquinoline. As it is necessary in any study of metabolism to have an understanding of the methods used for the quantitative measurement of ^{the} substance in question, the methods for measuring magnesium will be described in some detail.

2a. Gravimetric method. McCrudden (1911) adapted the method of precipitating magnesium as magnesium ammonium phosphate to the analysis of foodstuffs, urine and faeces. Magnesium is determined by ashing the magnesium ammonium phosphate precipitate which is then weighed as the pyrophosphate. Magnesium is expressed in terms of magnesium oxide. Many of the determinations in urine have been reported in this manner.

2b. The method has several defects. During the ashing procedure losses of magnesium may occur because of the formation of magnesium-

silica combinations which can only be broken down by fusing with sodium carbonate (Frear and Kahlenberg 1933). Instead of this ashing procedure, a method has been reported which allows the preparation of the original precipitate in a crystalline form which is then weighed (Washburn and Shear 1932).

2c. In the gravimetric method the precipitation of calcium constitutes the first step and it is necessary to know what technique is used for this, to be certain that none of the magnesium is precipitated with the calcium. To overcome this difficulty, Washburn and Shear (1932) have described a method of estimating calcium, magnesium and phosphate in the presence of each other. Their method appears to have a high degree of accuracy.

3. Molybdenum blue method. This technique is a modification of the previous McCrudden method. It is used for the determination of magnesium in body fluids and is based on the precipitation of magnesium as magnesium ammonium phosphate, with the subsequent colorimetric estimation of the blue compound which is formed by the interreaction of phosphate and ammonium molybdate (Briggs 1922, Denis 1922). Modifications have been presented by Godden and Duckworth (1935). However because of the instability of the blue color formed by this method many workers have found difficulty in obtaining consistent results. A further improvement has been reported recently by Simonsen et al. (1947) in which the phosphate is

estimated by the yellow color produced by addition of ammonium salts of vanadate and molybdate.

4. Hydroxyquinoline method. The use of 8-hydroxyquinoline to precipitate magnesium was first recorded by Fox (1910). It was adapted for the micro-estimation of magnesium in blood by Yoshinatsu (Duckworth, 1939). The method was converted to a titration procedure by Greenberg and Mackey (1932) and Velluz (1934) who used a bromination technique. However, Hoffman (1937) discarded the bromination method in favour of a colorimetric procedure based on the principle that 8-hydroxyquinoline combines with ferric ion in weak hydrochloric acid to form a specific green-blue color which was stated to be easy to determine in a colorimeter. Greenberg et al. (1933) and Eveleth (1937) found good agreement between the two modifications. The chief objection to the precipitation of magnesium as magnesium hydroxyquinoline is the flocculent nature of the precipitate which makes loss in handling likely to occur (Simonsen et al. 1947).

5. Titan yellow method. The titan yellow method is much less time consuming than the older ammonium phosphate and hydroxyquinoline methods. In addition, Duckworth (1939) has pointed out that in those methods manganese is precipitated with the magnesium and this introduces an error dependent on the concentration of the manganese. The titan yellow method is based on the fact that magnesium forms a red lake with titan yellow dye in an alkaline solution (Kolthoff 1927). Hirschfelder and Serles (1934) introduced a rapid method

based on this reaction. They used starch to produce a colloidal dispersion of the red lake and measured the density of the color by means of a micro-colorimeter. However, the resulting color was a mixture of pink and yellow and difficulty was encountered in the matching. These investigators claimed that the presence of calcium ions altered both the intensity and the nature of the color produced by magnesium, but Garner (1946) has published evidence to show that calcium, when present in a concentration comparable to that found in plasma, does not produce an error. Recently, Kunkel et al. (1947) modified the procedure by using hydroxylamine hydrochloride as the colloid dispersing agent for the red lake. It is this recent modification of the titan yellow method which was employed in the present study.

C. OCCURRENCE AND DISTRIBUTION OF MAGNESIUM.

1. General occurrence. The animal organism is dependent for its supply of magnesium on plants which, in turn, derive their mineral constituents from the soil. Magnesium is present in the earth's crust in the form of carbonate, sulphate, chloride, silicate and phosphate (Kendall 1936). The soluble chloride form is common, so plants usually have no difficulty in obtaining their supply. In plant juices magnesium is present as the salts of weak acids such as carbonic, lactic, citric, tartaric and malic (Schmidt and Greenberg 1935). It is present also in high concentration in

chlorophyll. Therefore, green vegetables are an important source of this element. Table I illustrates the approximate amounts of magnesium in some common foods. Because Duckworth has pointed out the disturbing effect of manganese in the older methods of estimation of magnesium the reliability of some of the values may be questioned.

2. Distribution in the animal body. Approximate concentrations of magnesium in various body tissues are illustrated in Table II. In animal tissues manganese exists only in small traces hence more confidence may be placed in values reported. This table is that used by Everett (1942a). He did not indicate whether the concentration of magnesium was expressed on a wet or dry weight basis. However, the table serves to indicate the relative proportions of magnesium in various organs. From the table, it is evident that bone and muscle contain the largest amounts of magnesium and in descending order of magnesium concentration: kidney, liver, pancreas, heart and brain. An interesting observation was made by Williamson and Gulick (McCance and Widdowson 1944). By chemical and physical methods they separated the nuclei from the cells of the thymus gland and analysed them for magnesium. The dried nuclei contained 0.9 per cent of magnesium.

3. Alteration with growth. The total amount of magnesium in the body has been most extensively studied in the rat. No effect of increasing age on the amounts of magnesium present in the body could

APPROXIMATE CONCENTRATIONS OF MAGNESIUM IN VARIOUS
COMMON FOODS

(Table compiled from data presented by Schmidt and Greenberg (1935) and Everett (1942).)

Foodstuff	Magnesium concentration in milligrams per cent
Beef	24
Eggs	11
Egg yolk	16
Milk	12
Cheese	37
Wheat	133
Potatoes	28
Corn meal	84
Oranges	12
Almonds	251
Spinach	37
Beans (dried)	156
Peanuts	180
Oatmeal	120
Chocolate	290

APPROXIMATE TISSUE CONCENTRATIONS OF MAGNESIUM*

Tissue	Magnesium concentration in milligrams per cent
Muscle	21.0
Skeleton	95.0
Kidney	20.0
Aqueous humor	2.5
Bile	0.5
Cerebrospinal fluid	3 ± 0.5
Lymph	3.0
Milk, human	5.0
Milk, cow	14.0
Saliva	2.0
Brain	14.0
Cartilage	11.0
Heart	16.0
Intestine	7.5
Liver	17.0
Lung	7.5
Pancreas	17.0
Skin	12.0
Spleen	15.0
Testis	9.5
Thyroid	9.5

* Taken from Everett (1942a)

be demonstrated by Buckner and Peter (1922) or Medes and Humphrey (1927). However, Greenberg and Tufts (1936) found a rapid increase in the percentage of body magnesium from birth up to four weeks of age. The percentage of magnesium then remained constant until about the eleventh week when it fell by one fifth. The work of Greenberg seems to be more convincing because his results form a smooth curve. Furthermore, it is supported by the findings of Hammett (1925) who reported that the percentage of magnesium in bone ash in rats increased at first and then decreased with increasing age. These results appear to indicate that there is an increase of the percentage of body magnesium during the period of body growth.

4. Bone. From Table II it is evident that magnesium in the body is concentrated in the skeleton, but it is also present in moderate amounts in other tissues. This is in contrast to calcium which has a low concentration in body tissues compared to the amounts in bone. Shohl (1939) has stated that the magnesium of bone forms a fairly constant fraction, constituting approximately 0.5 per cent of bone ash. It has been the general opinion that this fraction is in the form of magnesium phosphate and magnesium carbonate. Recent work on the composition of bone has indicated that the "salt" of normal bone is a complex mineral substrate (Everett 1942b), but that magnesium forms an integral part of this substrate has not yet been demonstrated.

5. Muscle. Muscle in contrast to bone contains much more magnesium than calcium (Shohl 1939), but in what form magnesium exists in muscle is in doubt. Sjollemma and Seekles (1933) have obtained muscle fluid by subjecting muscle tissue to pressures of 25 and 200 atmospheres. The magnesium concentration was 20 milligrams per cent. Ultrafiltrates from such muscle fluid contained 15 milligrams per cent. It seems probable then, that magnesium is present in muscle in an ionized state as well as in combination with some larger molecule such as protein which will not diffuse through a colloidion membrane. Schmidt and Greenberg (1935) quoting Lohman, state that a small amount of magnesium is combined with the adenylypyrophosphate present in muscle. Scott and Packer (1939) have demonstrated by means of the electron microscope that the magnesium content in muscle is very high and almost entirely in the cell. There is a small part in the tissue spaces and none in the sarco-plasm. They also found that muscular contraction caused a decrease in the muscle magnesium.

6. Red blood cells. In the blood, magnesium is present in both the red blood cells and the plasma. The red blood cells contain a higher concentration than the plasma. Studies on a large number of species have shown that except in ruminants, the concentration of magnesium in the red blood cells always exceeded plasma magnesium (Eveleth 1937). This is in contrast to calcium which is present

almost entirely in the plasma. Greenberg et al. (1933) found 6.6 milligrams of magnesium per 100 millilitres of red blood cells. Hald and Eisenman (1937) obtained the value of 4.6 milligrams per 100 millilitres. Recently Kunkel et al. (1947) obtained a mean value of 3.82 milligrams per 100 millilitres with a range of 3.58 - 4.50. This is less than the amount found by Greenberg but only six samples were examined and in addition the plasma levels were lower than the usually accepted values.

6b. The chemical state in which magnesium exists in red blood cells has not been determined. Schmidt and Greenberg (1935) have observed that a considerable fraction of the magnesium in laked red blood cells is not diffusible. Wolff (1939) demonstrated that the magnesium content of red blood cells was unaffected by washing the cells with physiological saline, and although the content may be increased by washing with a solution richer in magnesium, this increase is lost by subsequent washings in saline. An increase was found also following injections of magnesium in vivo. That author concluded that the magnesium normally in the cells must be combined with some non-diffusible molecule. He stated that isoelectric precipitation showed that it was not combined with hemoglobin.

7a. Plasma. There is some disagreement in the literature as to the normal level of serum magnesium in the human. The concentration of normal serum magnesium obtained by 26 different investigators

and the methods employed by each are shown in Table III. It is apparent from this table the range of serum magnesium levels of all the authors is 1.60 to 3.66 milligrams per cent. This range might suggest a wide variation in the serum magnesium concentration of humans but is probably due to inadequate analytic technique, because different authors using the same method obtained markedly different results. Most determinations of serum magnesium have been made using the ammonium phosphate method and as has been discussed this method has certain defects. However, the average of the maximum and minimum of all the ranges obtained by different methods is 1.83 to 2.76 and this can be taken as the approximate concentration of magnesium in human plasma.

7b. In plasma, magnesium exists in two forms commonly termed diffusible and non-diffusible. These are distinguished by the ability of the diffusible fraction to pass readily through membranes impermeable to colloids, while the non-diffusible fraction is held back by such membranes. About 70 per cent of serum magnesium is diffusible (Tschimber and Tschimber 1924, Benjamin et al. 1933, Watchorn and McCance 1937, Cope and Wolff 1942, Soffer et al. 1939, Bissell 1945, Schmidt and Greenberg 1935). It is the opinion of Hastings et al. (1934) and McLean and Hastings (1935) that the diffusible magnesium is entirely ionic. The non-diffusible portion is probably bound to protein in the same way that non-diffusible calcium is bound to serum proteins (Schmidt and Greenberg 1935).

CONCENTRATION OF NORMAL SERUM MAGNESIUM REPORTED BY DIFFERENT AUTHORS AND
METHOD USED BY EACH

Authors	Precipitating Agent	Method	Range of Values in mgm. %	Mean
Briggs (1922)	Phosphate	Colorimetric	2.23-2.50	
Cohen (1927)	Phosphate	Colorimetric	2.09-2.94	2.56
Kramer and Tisdall (1921)	Phosphate	Colorimetric	1.80-2.30	2.10
Kramer and Tisdall (1921a)	Phosphate	Colorimetric	2.10-2.90	2.60
Bogert and Plass (1923)	Phosphate	Colorimetric	1.90-2.70	2.30
Watchorn and McCance (1932)	Phosphate	Colorimetric	2.30-2.66	2.48
Salvesen and Linder (1924)	Phosphate	Colorimetric	1.70-1.90	1.80
Becher (1932)	Phosphate	Colorimetric	1.80-2.30	
Wacker and Fahrig (1932)	Phosphate	Colorimetric	2.00-2.97	2.40
Cope (1936)	Phosphate	Colorimetric	1.82-2.63	2.06
Walker and Walker (1936)	Phosphate	Colorimetric	1.60-3.00	2.20
Brookfield (1937)	Phosphate	Colorimetric	1.89-2.19	2.04
Bomskov (1932)	Hydroxyquinoline	Bromination	1.70-2.60	
Greenberg et al. (1933)	Hydroxyquinoline	Bromination	2.00-3.66	2.74
Velluz and Velluz (1934)	Hydroxyquinoline	Bromination	1.60-2.40	2.00
Raices (Haury, 1942)	Hydroxyquinoline	Bromination	1.69-3.00	2.44
Hoffman (1937)	Hydroxyquinoline	Colorimetric	1.90-2.50	2.18
Hirschfelder and Haury (1938)	Titan Yellow	Colorimetric	1.80-2.40	2.11
Haury (1940)	Titan Yellow	Colorimetric	1.70-3.10	2.33
Bernstein & Simkins (Haury, 1942)	Titan Yellow	Colorimetric	1.23-3.54	
Katzenelbogen and Snyder (1943)	Phosphate	Colorimetric	1.50-4.50	2.57
Soffer et al. (1939)	Phosphate	Colorimetric	2.12-2.76	2.52
Dine and Lavietes (1942)	Phosphate	Colorimetric	1.60-2.20	
Cope and Wolff (1942)	Phosphate	Colorimetric	1.75-2.45	1.99
Bissell (1945)	Phosphate	Colorimetric	1.91-2.68	2.28
Kunkel et al. (1947)	Titan Yellow	Colorimetric	2.02-2.22	2.14

8. Cerebrospinal fluid. Comparatively little work has been done on the estimation of magnesium in cerebrospinal fluid. Cohen (1927) found an average cerebrospinal fluid value of 3.28 milligrams per 100 millilitres, compared with an average serum value of 2.56. This work was confirmed by McCance and Watchorn (1931) who obtained a mean value of 3.33 milligrams per 100 millilitres in sixty cerebrospinal fluid levels of magnesium with serum levels in the same patient. They concluded that the concentration of magnesium in the cerebrospinal fluid was remarkably constant irrespective of the concentration in the serum.
9. Other body fluids. The concentration of magnesium in human amniotic fluid is only one tenth of that of plasma (Schmidt and Greenberg 1935). This is remarkable in view of the fact that cattle show similar concentrations of magnesium in plasma and amniotic fluid (Duckworth 1939). Human gastric juice has been found to contain magnesium in amounts from 0.74 to 8.8 milligrams per cent, with high values sometimes associated with low acidity (Sendroy 1945). Because of the importance of milk in animal nutrition, it is noteworthy that cows milk contains about three times as much magnesium as does human milk (See Table II). Published data on the magnesium content of transudates and lymph are scarce. Greene et al. (1931) have reported a series of ten observations made on human ascitic and pleural fluids. They found a mean of 2.2 milligrams per 100 milli-

litres but the protein content of the fluids averaged 3.1 grams per cent. Ascitic fluids that are produced experimentally in dogs and which have a low protein content have amounts of magnesium present which are in good agreement with those found in ultrafiltrates of serum of the same dogs. This agreement supports the conception that such fluid is a simple dialysate. With increasing amounts of protein in the fluid, the quantity of magnesium is increased.

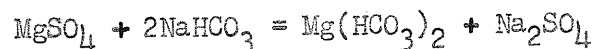
D. METABOLISM OF MAGNESIUM

1. Definition. Metabolism has been defined by Best and Taylor (1945) as "the term employed to embrace the various chemical processes occurring within the tissues upon which the growth and heat production of the body depend and from which the energy for muscular activity and for the maintenance of vital functions is derived". In the present account the term "magnesium metabolism" has been used in this broad sense to include the manifold activities of magnesium in life processes.
2. Absorption. If the amount of magnesium excreted in the urine is taken as an index of absorption, a number of soluble magnesium salts seem to be readily absorbed, particularly citrate (Bogert and McKittrick 1922), lactate (Carswell and Winter 1931, Tibbetts and Aub 1937a), and chloride (Taylor and Winter 1929). Magnesium

chloride administered by mouth in doses of 200 milligrams per kilogram body weight nearly doubled the concentration of serum magnesium of dogs (Winter and Rickey 1931). The oral administration of magnesium sulphate in a single dose of 20 to 40 grams does not change the plasma magnesium significantly in normal persons, although approximately 40 per cent of the magnesium is absorbed and excreted in the urine within 24 hours (Hirschfelder 1934). Hirschfelder (1934) showed that after giving magnesium sulphate in the above amounts slow absorption occurred but that it was excreted so rapidly by the kidneys that the plasma level of magnesium was not affected. To demonstrate this, he conducted experiments on rabbits with kidney damage, and found that a marked increase in serum magnesium occurred 3 to 4 hours after the ingestion of 2 grams of magnesium per kilogram body weight. When ordinary laxative doses of 2 to 10 grams of magnesium sulphate are used very little absorption of magnesium occurs because the degree of absorption depends on the time of sojourn in the intestines (Sollmann 1942). Because of the marked hydrogogue effect of magnesium sulphate it passes through the intestine and reaches the colon in $1\frac{1}{2}$ to 2 hours. Thus, there is not sufficient time for much magnesium to be absorbed.

2b. In the stomach, magnesium oxide, hydroxide, and carbonate are first partly converted into chlorides by the gastric acid. According to Shohl(1939a) magnesium is liberated also from its combination

in chlorophyll by the action of the acid in gastric juice. Sollmann (1942a) has stated that magnesium is absorbed chiefly from the small intestines. During their passage through the intestines, magnesium salts are mainly changed to the soluble bicarbonate by the reaction:



Absorption does not appear to depend on the presence of an acid or basic reaction in the intestine, since addition of 2 per cent of sodium carbonate to the diet of rats did not affect utilization (Duckworth 1939) nor was absorption affected by the addition of hydrochloric acid to the diet of dogs (Givens 1918). Recently McCance et al. (1942) have shown that proteins in the diet facilitate the absorption of magnesium salts.

3. Excretion. After absorption magnesium is excreted largely in the urine. It was shown by Magnus - Levy (Shohl 1939a) that as much as 90 per cent of magnesium injected intravenously is excreted by the kidneys. This has been confirmed by Nicolaysen (1936). When ingested by mouth a large proportion of excreted magnesium is found in the faeces. Tibbetts and Aub (1937a) demonstrated that in medical students who were receiving 300 milligrams of magnesium in their daily diet, one third of the magnesium was excreted in the urine and two thirds in the faeces. This distribution in the excreta resembles that of calcium. When extra magnesium was ingested in the form of magnesium lactate, it was excreted mainly by the

intestine but the urinary excretion also increased to as much as six times the control level, indicating some absorption. In a group of normal controls these authors found the 24 hour urine to contain 0.29 grams of magnesium. This value is in agreement with that accepted by Sollmann (1942a) and Best and Taylor (1945a).

4a. Magnesium balance. Studies of mineral balance in the animal organism should include a measurement of the intake and excretion of the mineral concerned. The calculation of the resulting balance indicates whether the body is in positive or negative balance, or in equilibrium. It has been considered by Leitch (1937) that the intake is adequate when the results demonstrate an equal number of cases of small positive and negative balances. It is important that sufficiently long control periods be observed, in order to determine this adequate intake. Furthermore, food must be analysed by the same methods as used for faeces and urine. The use of average values given in food tables is not accurate enough for balance studies in individual cases. Much of the work done on magnesium balance fails to fulfil these criteria but a thorough study of magnesium balance in humans has been carried out by Tibbetts and Aub (1937, 1937a, 1937b). Their results have been used as the basis for the following discussion of magnesium balance.

4b. These authors found that on a neutral low-calcium diet an intake of 220 milligrams of magnesium per day resulted in essentially

positive balances in hospital patients. Medical students who were pursuing their normal activities stored magnesium on an intake of 300 milligrams. The ingestion of extra magnesium in the form of magnesium lactate did not cause an extra storage of magnesium in normal subjects. The excess was merely excreted both in urine and faeces. However, this added magnesium did increase the excretion of calcium in the urine. The addition of phosphate or calcium to the diet produced no marked effect on magnesium excretion. Though acid ingestion increased the total excretion of magnesium it did so to a lesser extent than it affected calcium excretion.

4c. Little difference in the magnesium balance from that of normal controls was found in hyperparathyroidism, exophthalmic goitre, Cushing's disease, Addison's disease, steatorrhea, and pituitary basophilism. Following parathyroidectomy there is a temporary fall in magnesium excretion into the urine but this returns to pre-operative levels within 2 months. It is remarkable that in these conditions which markedly disturb calcium balance there is little change in the balance of magnesium. The results would appear to indicate that magnesium may be more independently metabolized than has been recognized heretofore. Certainly, from these studies it can be concluded that magnesium, calcium, and phosphorus do not respond as a group.

5a. Relation to calcium. The fact that high magnesium intakes usually have the effect of depleting the bones of calcium and increasing the urinary excretion of calcium, indicates some direct action on calcification. The way in which this is accomplished is not clear. Recent evidence has revealed that a decrease in serum magnesium, resulting from a magnesium deficient diet, is accompanied by a marked fall in serum phosphatase by magnesium has been demonstrated (Jenner and Kay 1931). Since the interrelationship of phosphatase and calcium with regard to bone formation has been established (Kay 1932) the evidence suggests that magnesium may be linked with the process of calcification through its effect on phosphatase activity.

5b. Studies of the effect of magnesium on calcium balance and on the occurrence of skeletal changes appear to fall into two groups. Some indicate that increased intake of magnesium favors calcium retention (Schuler, Euler and Rydbom, Becka, quoted by Duckworth 1939, Barbour and Winter 1931, Carswell and Winter 1931). Others give evidence to show that depletion of calcium results (Buckner et al. 1932, Villius 1932, Tibbetts and Aub 1937, Hart and Steenback 1913). Duckworth (1939) has concluded from this contradictory evidence that "under certain circumstances, the addition of magnesium to the diet has increased calcium retention, while, on the other hand, increases in dietary magnesium certainly results in a

tendency to decalcify the skeleton". However, McCance et al. (1942) have shown that proteins facilitate the absorption of magnesium. Therefore, it seems more likely that the disagreement of results can be explained on the failure to realize the importance of protein in the diet in regard to the absorption of magnesium. Another factor which might affect the results is the type of anion combined with the added magnesium. For example, magnesium chloride may act differently than magnesium carbonate. Only when all these factors are considered and experimentally controlled will it be possible to evaluate the effects of magnesium on the growth of bone. A finding which may have far reaching implications not only with respect to bone metabolism but also to the state of calcium and magnesium in the fluids of the body, is the discovery of Dickens (1941) that in several species of common domestic and laboratory animals as much as 70 per cent of the body content of citrate is present in the hard substance of bone. This discovery has been confirmed by Thunberg (1943) and Martensson (1943). The possible interrelationships of citrate with calcium and magnesium in the bony salts may furnish a new field of investigation.

6a. Relation to carbohydrate metabolism. The production of hyperglycaemia and glycosuria by the injection of magnesium salts was first noted by Meltzer and Auer (Schmidt and Greenberg 1935). Underhill and Closson (1905) believed that the increase in blood

glucose was due to asphyxia. But Kleiner and Meltzer (1915) by using artificial respiration showed this was not so. Duckworth (1939) has stated that Hazard and Vaille thought that the action of magnesium was due to stimulation of the sympathetic system and not to increased output of adrenaline but Corkill and Ennor (1938) were unable to produce hyperglycaemia by magnesium after adrenalectomy.

6b. The absorption of glucose from the intestine has been shown to be decreased by the injection of magnesium gluconate (Ludany and Suto-Nagy 1940). The injection does not influence the absorption of xylose. However, serum inorganic phosphate may be increased up to 100 per cent. These authors have provided the explanation that magnesium may withdraw phosphate from the tissues and this lack of tissue phosphate reduces glucose absorption. Xylose absorption which is not associated with phosphorylation is unaffected.

7. Relation to vitamins. Although vitamin D increases the absorption of calcium, no similar effect has been shown to exist with regard to the assimilation of magnesium. Huffman and Duncan (1935) have reported that magnesium reduced the quantitative need for vitamin D in calves, but did not prevent rickets when added as a supplement to a rachitogenic diet. Furthermore, Swanson (1932) demonstrated a decreased retention of magnesium when cod liver oil was administered to an infant which was fed cows milk. Magnesium retention was said to be improved in children (Chaney and Blunt 1925),

and also in young girls (Chaney and Blunt 1926) by the administration of orange juice. Bogert and Trail (1922) have reported that yeast improved the magnesium balance of young women but the results may not have been due to vitamin B as the effective agent might have been some other constituent of yeast. It is evident therefore, that very little is known regarding the interrelationship of vitamins and the metabolism of magnesium.

8a. Activation of enzymes. There has been sufficient evidence accumulated to indicate that magnesium acts as an activator of certain enzymes, particularly phosphatase and phosphorylase. Phosphatases occur in all tissues of the body but particularly in teeth, bone, kidney, intestinal mucosa, liver and to a smaller extent in blood. In bone and teeth this enzyme can hydrolyze hexose phosphates liberating inorganic phosphate. It appears to play an important role in the deposition of calcium phosphate in growing bone (Cameron 1945). The kidney and intestinal mucosa contain non-specific phosphatases which hydrolyze glycerophosphate, hexose diphosphate and muscle adenylic acid and are capable of acting on nucleotides (Cameron 1945a). In the liver glycogen is broken down to glucose-1-phosphate. A phosphatase in the liver splits this to form phosphate and free glucose. All these reactions have been shown to be either activated or accelerated by magnesium ions (Jenner and Kay, 1931, Duckworth 1939). The action of magnesium is

not specific as manganese also may activate these reactions (Everett 1942c). Nevertheless, that magnesium is associated with serum phosphatase activity has been recently reported by Snyder and Tweedy (1942), and Fischer and Greep (1948).

8b. Phosphorylation is a process concerned with the absorption of fat and sugars from the intestine and is necessary for the formation of many active compounds in animal tissues. Cameron (1945b) states it must be one of the commonest biological reactions. With regard to the absorption of sugars it has been suggested that the relatively rapid rate of absorption of glucose, galactose and fructose is dependent on phosphorylation (Beck 1942), which results in the formation of hexose phosphates. Fats are converted to phospholipids by the process (Canterow and Trumper 1945). Phosphorylation is also an important phase of carbohydrate catabolism (Everett 1942c). This process of phosphorylation is accomplished in animal tissues by phosphorylase which is said to be activated by either magnesium or manganese (Everett 1942c). Lohman (Duckworth 1939) found that in mammalian muscle, hydrolysis of adenyolphosphoric acid proceeds by two steps and that dephosphorylation occurs only in the presence of magnesium. Magnesium ions have also been found to function as activators of the transfer of phosphorus in the synthesis of adenosinetriphosphoric acid in mammalian muscle (Ostern and Baranowski 1935).

8c. Apparently all animal tissues, including liver and muscle contain a specific enzyme phosphoglucomutase which converts glucose-1-phosphoric acid to glucose-6-phosphoric acid. Cori et al. (1939) have found that magnesium greatly accelerates the activity of this enzyme.

8d. The enzyme carboxylase is widely distributed in animal tissues. According to Cameron (1945c) it brings about the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide. This reaction is intimately associated with many oxidation - reduction systems and may be the most important way in which carbon dioxide is produced in the living cell. Carboxylase has been isolated from brewer's yeast as a diphosphothiamine-magnesium-protein (Green et al. 1941).

8e. From the above evidence it is apparent that magnesium plays an important part in many enzyme reactions. It acts as a co-enzyme in the process of phosphorylation. It activates various phosphatases which are present in animal tissues and is an actual constituent of carboxylase.

E. PHARMACOLOGY OF MAGNESIUM

1. Effect on intestine. It is the general opinion that magnesium sulphate when used as a laxative produces catharsis by retention of fluid in the intestine. The retained fluid acts as a

distensive stimulus to the intestine. Magnesium also exerts a depressant action on intestinal muscle but this effect is overbalanced by the mechanical stimulus of distension (Sollmann 1942b).

2. Toxicity from intestinal absorption. Auer and Meltzer (1913) demonstrated that a 20 per cent solution of magnesium chloride introduced into the duodenum of cats was lethal. The animals died after 25 minutes with respiratory paralysis but without convulsions. Very few cases of human poisoning by oral administration have been reported. Fraser (1909) reviewed the literature from 1841 and was able to collect only seven cases. Boos (1910) reported ten cases. Byron has reported that five children with intestinal worms all died following the administration of magnesium sulphate by mouth (Fawcett and Gens 1943). Autopsy findings in a case of magnesium poisoning, have been reported by Thatcher (1928). These consisted of (a) hemorrhagic appearance of gastric mucosa, (b) recent hemorrhages throughout the small intestine, (c) marked congestion of lungs, trachea, main bronchi, heart, liver and kidneys. These findings are rather non-specific and tend to support the idea that these cases are due to idiosyncrasy. Fawcett and Gens (1943) have reported two cases of magnesium poisoning following an enema of epsom salt solution. They state that serum magnesium was increased. However, some doubt can be cast on this because one of the cases only retained the enema for 2 minutes which is obviously insufficient time for much absorption to occur. It seems possible that absorption of

magnesium resulting in high serum magnesium and toxic effects could occur if the kidneys were damaged (Hirschfelder 1934) or if intestinal obstruction was present. This may be the explanation for the death of the five children with intestinal worms. But because estimations of serum magnesium were not done, definite conclusions cannot be made. At any rate, the available evidence offers little proof that poisoning by magnesium does occur.

3a. Effect on the heart. The injections of large doses of magnesium sulphate intravenously produces an immediate and often fatal depression of the heart and central nervous system. A fall in blood pressure is the most constant first result. It occurs before the serum concentration reaches 6.1 milligrams per cent. The blood vessels are markedly dilated and the pulse rate rises. Arrest of respiration and the heart occur almost simultaneously when the serum magnesium concentration reaches 18 to 31 milligrams per 100 millilitres. With abrupt injection the heart may be arrested instantly (Hoff et al. 1939, Smith et al. 1939, Moore and Wingo 1942). Cardiac effects consist of slowing with moderate dilatation, then incoordination and finally standstill (Zwillinger 1935). There is no general agreement about the mechanism of this action. Zwillinger (1935) observed that paroxysmal tachycardia was stopped by the intravenous administration of the drug but auricular fibrillation and flutter were not influenced. Rothberger and

Zwillinger (1936) showed experimentally that magnesium salts combated ventricular tachycardia produced by barium chloride and strophanthin. The slowing effect of intravenous magnesium sulphate has been demonstrated to consist of a delay in the auriculoventricular and intraventricular conduction and is associated with an increased excursion of all complexes (Miller and Van Dellen 1941). Smith et al. (1939) showed in dogs that after a transient tachycardia, bradycardia appeared and there was a progressive increase in the P-R interval as the concentration of magnesium increased until A-V block and widening of QRS appeared. Bernstein and Simkins (1939) injected rapidly 10 millilitres of a 10 per cent solution of magnesium sulphate into 34 non-cardiac patients and found minor changes in the "T" waves and QRS complexes in six only. They concluded that the intravenous injection of magnesium sulphate in such doses exerted no deleterious effect on the normal human heart.

3b. The intravenous injection of magnesium has been recommended as a useful therapeutic procedure in paroxysmal tachycardia by Boyd and Scherf (1943). They succeeded in terminating each of eight attacks. Szekely (1946) reported that of thirteen attacks of paroxysmal tachycardia nine were either completely abolished or altered while extrasystoles from other causes were not affected by it.

4. Effect on vascular system. It has been demonstrated conclusively that magnesium is a powerful vasodilator (Hoff et al. 1939, Winkler et al. 1942, Haury 1939, Haury 1939a). It produces flushing, sweating and a sensation of warmth in man (Winkler et al. 1942). If small amounts are used a fall in blood pressure does not occur. However, if large doses are administered there is a gradual fall in blood pressure in the anaesthetized dog (Hoff et al. 1939) and often a sudden drop in normal unanesthetized man (Winkler et al. 1942). The fall in blood pressure depends upon the rate of injection and the size of dose. Hypertensive subjects frequently do not show any fall in blood pressure (Winkler et al. 1942).

5a. Effect on nervous system. In 1905 Meltzer and Auer (Sollmann 1942c) discovered that the subcutaneous injection of magnesium sulphate in doses of 1.5 grams per kilogram of body weight produced a general anaesthesia with abolition of the reflexes. The anaesthesia was complete in half an hour and lasted about 2 hours. Peck and Meltzer (1916) produced anaesthesia in three human subjects and showed that the effect was central. However, the effective dose approaches the fatal dose so closely that this method is unsatisfactory for use in man. Intramuscular injections of magnesium sulphate have been used in obstetrical anaesthesia to reinforce rectal ether-oil anaesthesia (Gwathmey 1925). However, the dosages used do not raise the serum magnesium above 3 milligrams per cent

and since no symptoms of depression appear below 3 milligrams per cent there is no proof that the injections are of any use (Neuwirth and Wallace 1929). Beckman (1925) has concluded that there is no proof of any potentiation of ether or morphine by magnesium. He believes the action is simply additive. The nature of the anaesthetic action of magnesium is now believed to consist of a true, abolition of sensation by a central effect (Peck and Meltzer 1916) and a paralysis of muscular response by a curare-like action (Sollmann 1942c).

5b. The curare-like effect was discovered by Jolyet and Cahours in 1869 (Sollmann 1942c) and has been confirmed by many other workers. Like curare it antagonizes twitchings produced by physostigmine and its curare effect is, in turn, abolished by calcium, potassium and acetylcholine. However, the resemblance to curare is not complete for the threshold of stimulation is increased also in denervated skeletal muscles of dogs (Maaske and Gibson 1939). It is now recognized that ionic concentrations exert an important influence upon neuro-muscular irritability. An increase of sodium or potassium ions enhances whereas calcium, magnesium, and hydrogen ions diminish this excitability, (Duncan 1947a). The relationship is expressed as follows:

$$\text{Irritability} \propto \frac{(\text{Na}^+) + (\text{K}^+)}{(\text{Ca}^{++}) + (\text{Mg}^{++}) + (\text{H}^+)}$$

Since calcium and magnesium act in a similar manner in this regard it seemed peculiar that the depressant action on neuro-muscular transmi-

ssion, produced by the parenteral injection of magnesium salts was relieved by administration of calcium (Peck and Meltzer 1916). Recently, Dubois et al. (1943) have postulated that in the animal anaesthetized with magnesium, the muscles are unable to contract because calcium is blocked from adenosine triphosphatase by the chemically similar magnesium ion. Because calcium has been shown to be a specific activator of adenosine triphosphatase (Bailey 1942) and magnesium inhibits this reaction, they believed that a much higher yield of adenosine triphosphate should be obtained from the muscles of a magnesium treated animal than from a control animal. They found this to be so and suggested that in magnesium anaesthesia the magnesium ion may compete with the calcium ion for the surface of adenosine triphosphatase and thus prevent a breakdown of adenosine triphosphate.

5c. Meltzer and Auer have claimed that concentrated solutions of magnesium sulphate act as efficient local anaesthetics (Sollmann 1942c). The subdural injection of magnesium was also carried out by these workers. They used one millilitre of a 25 per cent solution and produced sensory and motor paralysis of the legs and pelvic region lasting 8 to 14 hours. This procedure has been advocated for the relief of intractable pain by Moore (1941) and Stokes (1934). However, serious results have occurred following its use (McKendree 1940, Guttman and Wolf 1944).

6a. Effect on liver and gall bladder. It was suggested by Meltzer (1917) that if a strong solution of magnesium sulphate was introduced into the duodenum it would cause a relaxation of the sphincter of Oddi because magnesium solutions were known to relax intestinal muscle (Meltzer 1915). Lyon (1920, 1922) made use of this suggestion by introducing 40 millilitres of a 33 per cent solution into the duodenum and collecting the bile at intervals. The first samples were light yellow which he presumed to come from the common ducts. Then followed a darker and more viscous bile which he believed came from the gall bladder. Finally a light thin bile was obtained which was of low specific gravity. Lyon called these different samples of bile A, B and C bile. He believed that "B" bile reflected the condition of the gall bladder and that repeated "drainage" by this means would be beneficial in cholecystitis. In recent years warm olive oil has supplanted the use of magnesium sulphate as a cholagogue.

6b. Several workers have challenged the belief that "B" bile was necessarily from the gall bladder. Bile flow has been shown to be increased even if the gall bladder was excised (Dunn and Connell 1921) or if the cystic duct was ligated (Mendenhall et al. 1926). Furthermore, Dunn and Connell (1921) demonstrated that A, B and C types of bile could be collected after the instillation of magnesium sulphate in the duodenum of a patient with a

hepatoduodenostomy and no gall bladder. These workers also showed that the concentration of magnesium in the bile varied with the concentration of the bile pigments. They concluded that the different colors of A, B and C bile were due to the amount of absorbed magnesium excreted in the bile. Matsuo (1924) observed the contraction of the gall bladder at operation after the introduction of magnesium sulphate into the duodenum. By the use of red dyes he demonstrated that some of the increased amount of bile came from the gall bladder at operation after the introduction of magnesium sulphate into the duodenum. By the use of red dyes he demonstrated that some of the increased amount of bile came from the gall bladder. Silverman and Menville (1925) reported observations on the visualized gall bladder which they state contracted following the administration of magnesium sulphate. However, they only had 2 cases both of which were ill. In addition, the X-ray pictures which they show are not convincing with respect to any marked change in size of the gall bladder before and after magnesium sulphate. An explanation for the variance of the above findings was published by Gantt (1930). He stated that the expulsion of bile from the gall bladder after the instillation of magnesium into the duodenum was associated with vomiting or retching. Dogs which did not show retching did not produce an excess excretion of bile. He has reasoned that since magnesium sulphate causes a relaxation

of the sphincter of Oddi, vomiting which may be produced readily by passage of the tube alone or by the injection of substances into the duodenum, may cause expulsion of bile from the gall bladder into the duodenum.

7. Miscellaneous effects. The effect of injection of magnesium on the production of hyperglycaemia and glycosuria has been noted while its relation to body temperature is discussed on page 58. The production of hyaline casts in the urine following the injection of magnesium sulphate has been reported by Gates (1913) and Peck (1913). Magnesium salts also increase the acidity of the urine (Sollmann 1942c). Vomiting sometimes occurs during the intravenous administration in man (Winkler et al. 1942). It is commonly associated with an acute fall in blood pressure but the mechanism of the action is not known. A condition known as metal fume fever is produced by the inhalation of magnesium oxide fumes. In this condition there is a rise in body temperature, an increase in leukocyte count and general malaise. Recovery usually follows in 24 hours (Drinker et al. 1927).

8a. Use in eclampsia. The intravenous use of magnesium has been used by many clinicians for the treatment of eclampsia (Alton and Lincoln 1925, Dorsett 1926, Lazard 1925, Lazard 1933, Lazard et al. 1926, McNeil and Vruwink 1926, McNeille 1934). There seems no doubt that convulsions can be controlled by this method, but the mode of

action of magnesium in this respect has not been proved. However, because the vascular system is abnormal in eclampsia it is possible that magnesium exerts its effect by its vasodilator action. This seems to be particularly likely since a fall in blood pressure often occurs with clinical improvement. The depression of neuromuscular transmission by magnesium may also play an important role in controlling convulsions associated with vascular disease.

8b. Certain precautions should be observed when magnesium salts are injected intravenously. Smith et al. (1942) have found an isotonic or slightly hypotonic solution of about 2 per cent of the hydrated salt, $MgSO_4 \cdot 7H_2O$ both safe and effective in controlling convulsions. They administered approximately 500 millilitres of this solution in a period of 30 to 60 minutes. The disappearance of the tendon reflexes preceded any serious depression of respiration (See Table IV). Therefore, this is a valuable guide for determining over-dosage. Calcium chloride should be ready for immediate use in cases of sudden respiratory failure and it should be injected slowly to avoid the production of ventricular fibrillation (Hoff et al. 1939). Repeated injections of magnesium may be given every 4 or 5 hours if renal function is normal because the magnesium is rapidly excreted. However, in patients with diminished kidney function the concentration of magnesium in the serum may be elevated for several days. Therefore, repeated injections must be given with caution.

9. Relation of effects to concentration of magnesium in serum.

The various effects which are produced when the concentration of magnesium in the serum is at different strengths have been summarized in Table IV. This table has been reproduced from Smith et al. (1942) except the concentration of serum magnesium has been expressed in milligrams per cent.

F. MAGNESIUM DEFICIENCY

1. Content of deficient diets. It appears that the first attempt to deprive an animal of magnesium was made by Osborne and Mendel (1918). However, the diet they used contained 100 parts of magnesium per million and no untoward effects were apparent. Leroy (1926) gave mice a diet containing 10 parts of magnesium per million and found that growth ceased after 9 to 13 days and that three out of five of the mice died in 26 to 35 days. Finally, a state of acute magnesium deficiency was produced by Kruse et al. (1932) who used a diet containing 1.8 parts magnesium per million. The failure of the earliest workers to produce marked clinical changes was due presumably to the higher magnesium content of their diets.

2a. General signs of deficiency. The chief characteristics of magnesium deficiency in rats are: vasodilatation, hyperaemia of the cutaneous vascular system, increasing irritability, cardiac arrhythmia and fatal tonic-clonic spasms (Kruse et al. 1932).

TABLE IV

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EFFECTS OF PARENTERAL MAGNESIUM AND THE CONCENTRATIONS OF MAGNESIUM IN
SERUM AT WHICH THEY ARE USUALLY MANIFEST

Action	Magnesium of Serum in milligrams per cent
No apparent effect	2.4
Initial Tachycardia (dogs)	2.4 - 6.1
Initial Bradycardia (man)	2.4 - 6.1
Initial fall in blood pressure(dogs)	2.4 - 6.1
Flushing, sweating, sensation of heat	2.4 - 6.1
Vomiting	2.4 -12.2
Progressive fall in blood pressure	6.1
Beginning auriculo-ventricular block	12.2
Failure of tendon reflexes	12.2
Beginning intraventricular block	14.6
Failure of respiration	18.2
Failure of corneal reflex	36.5
Cardiac arrest	36.5

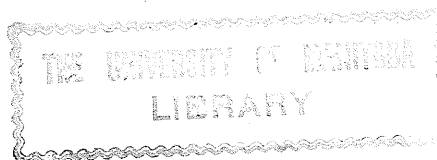
The animals usually die in the first convulsive attack but if they survive they develop trophic changes of the epidermal structures and edema of the extremities. Similar changes were found to occur in the dog but the trophic changes were more marked. According to Tufts and Greenberg (1938) there are two phases of physical change in magnesium deficiency. In the first place the chief manifestations are vasodilatation, hyperexcitability and tetany, and in the second, malnutrition, cachexia and kidney damage. These effects have been observed in varying degrees by most observers. However, these authors did not observe epidermal changes in animals on very low magnesium intakes. They have suggested that the diet used by Kruse et al. (1932) may have been low in vitamin B₂.

2b. Using a diet containing 40 parts magnesium per million Watchorn and McCance (1937) studied subacute deficiency in the rat. This diet permitted almost normal growth until the end of the 12 week experimental period when the appetite began to fail. During the early phase of deficiency it was observed that piebald rats were prone to diarrhea and melena. This was followed by hyperaemia of the skin and loss of hair. After a few days on the diet a characteristic smell became evident. Later a sticky, reddish exudate appeared on tails, paws and ears. This was evident particularly on albino rats. A similar result was obtained in vitamin B₂ deficiency but not nearly so pronounced. However, since the

control animals showed no such reaction it was thought that an interrelation existed between vitamin B₂ and magnesium.

3. Effect on growth. There is little retardation of growth during the first phase of magnesium deficiency but a marked decrease is evident during the second phase (Tufts and Greenberg 1938). When the diet contained 50 milligrams of magnesium per 100 grams of food, Tufts and Greenberg (1938) found that normal growth was maintained but they later demonstrated that female rats on this diet gave birth to young with less total body magnesium than the controls. It appears that the intake of calcium is an important factor in this regard. If the diet contained 0.9 per cent of calcium, the rats grew normally and produced young with normal total amounts of body magnesium on an intake of only 5 milligrams of magnesium per 100 grams of food. However, on high calcium intakes the minimal requirement of magnesium was increased. This is supported by the observations of Duckworth and Godden (1941) who demonstrated that rats survived longer and that vasodilatation was delayed and diminished on low calcium intakes.

4. Effect on body content of calcium and magnesium. Using dogs on a magnesium deficient diet Kruse et al. (1934) claimed that calcium was retained during the early stages of deficiency and increasingly eliminated during the later stages. However, they did



not measure intakes of calcium so no definite conclusions should be made from this work. Tufts and Greenberg (1938) also found that the percentage calcium content of the whole body was increased during the early stages of magnesium deficiency. The total body magnesium showed no decrease but since growth continued the percentage magnesium content of the body decreased. It appears then, that magnesium was retained as the deficiency progressed. This is supported by the work of Nicolaysen (1936) who demonstrated that in deficient dogs urinary excretion of magnesium decreased from about 8 to 2 milligrams per day over a period of 30 days.

5a. Effect on epidermal structures. As has been described the rats of Watchorn and McCance (1937) had loss of fur and a sticky reddish exudate on the paws, ears and tails. The exudate contained approximately 4 per cent phosphorus and seemed to consist partly of a keratin-like protein and partly of a lipoid. Tufts and Greenberg (1938) observed a similar exudate on their animals but did not notice either dermatitis or loss of hair. Schrader et al. (1937) have reported that a severe erythema followed by hemorrhagic purpura and then eschar formation was produced by a diet of acid-extracted yellow corn, wheat middlings, casein and a supplementary salt mixture. However, these workers did not state the magnesium content of the diet.

5b. It is evident that these reports concerning the pathological involvement of the skin are somewhat inconsistent. It seems probable that deficiency of the vitamin B complex is a complicating factor and may be responsible for the differences in results concerning changes in the skin. The alcoholic extract of yeast which is commonly used as a vitamin B supplement is apparently low in both riboflavin and vitamin B₆. It should be pointed out also that when the extract is mixed with the basal ration an inadequate intake of vitamin may result because the animals eat less as magnesium deficiency is prolonged. However, because the controls are reported to be free from pathological changes during investigations of magnesium deficiency, it seems that some of the effects may be due to the concurrent lack of magnesium and vitamin B. Greenberg (1939) has reported that skin lesions produced by magnesium deficiency could be retarded but not eliminated entirely by supplementing the low magnesium diet with crystalline thiamine hydrochloride, riboflavine and an 85 per cent alcoholic extract of a rice-bran preparation. On this diet, skin changes did not begin to develop until 35 days had elapsed.

6. Effect on the blood. In dogs the serum magnesium shows a decrease in concentration within a few days after the animals are placed on a diet deficient in magnesium (Kruse et al. 1933). In rats both plasma and red corpuscles also show an early decrease in

concentration of magnesium. The plasma magnesium decreases to levels of less than one milligram per cent and rises again to a peak shortly after the onset of the stage of hyperexcitability. Thereafter, there is a gradual decline (Tufts and Greenberg 1938). The magnesium content of the red blood cells is reduced to about half the normal value and remains about the same during the period of deficiency. Hoobler et al. (1937) measured the amount of diffusible magnesium in the plasma of magnesium deficient dogs and found it to be extremely low. They believe that the tetanic syndrome of acute magnesium deficiency is associated with the low concentration of magnesium ions in the blood. No changes in the inorganic phosphorus, lipid phosphorus, erythrocytes, carbon dioxide-combining power, fibrinogen, albumin, globulin, sodium, potassium, creatine, creatinine or glucose of the blood of dogs were observed by Kruse et al. (1933), although in rats a marked decrease of the serum proteins occurred and was associated with the development of edema of the extremities. The serum calcium of deficient rats remained unchanged from normal controls (Watchorn and McCance 1937) and no difference from normal levels was found in the phosphatase content of plasma. Recently, however, Snyder and Tweedy (1942) have shown that a decrease in serum magnesium, produced by a diet deficient in magnesium, is associated with a marked decrease in serum phosphatase activity.

7. Effect on bone. Grent et al. (1934) claimed that the bones of rats fed on a magnesium deficient diet contained a larger propor-

tion of ash than those of control animals fed on an adequate diet. However, Duckworth (1939) has pointed out that their data may be questioned because the sum of their values for calcium, magnesium and phosphorus (as oxides) accounted for far less of the bone ash than the totals accepted by other workers. Watchorn and McCance (1937) found that in rats the bones of the deficient animals contained more water and less magnesium than the controls. The magnesium in the bones of deficient rats was less than two thirds the normal values. On the other hand, calcium and phosphorus of the ash showed practically no change. It has been noted by Duckworth et al. (1940) that a diet containing 6 parts per million of magnesium resulted in decreased calcification of the bones as shown by a lower ash content. The magnesium content of the bones decreased rapidly during the first 6 days then at a much slower rate. These workers suggest that the two phase nature of magnesium deficiency may be explained on the basis of an initial rapid mobilization of the skeletal magnesium reserves for the use of the soft tissues. Later, this mobilization ceases.

8. Effect on teeth. Watchorn and McCance (1937) observed that the teeth of rats seemed to be more susceptible to the effects of magnesium deficiency than bone. In teeth the magnesium was only half of the normal value. They also observed striations in the dentine. Klein et al. (1935) found marked changes in the membrane lining

the roots of the teeth and in the adjacent alveolar bone. There was gross proliferation of the paradontal tissues and abnormal deposition of calcium salts along the borders of the alveolar bone.

9a. Effect on the kidney. Evidence of kidney damage has been reported by various workers. Greenberg et al. (1938) observed the development of albuminuria and a nephrotic type of kidney disease in their animals with magnesium deficiency. There was an increase in the volume of urine and in the excretion of protein but blood or casts were not present. Calcification of the cortico-medullary zone, the pyramids, and finally of the cortex was observed. Watchorn and McCance (1937) using a diet containing a greater amount of magnesium observed some kidney damage in rats but no albuminuria. Swanson et al. (1936) observed kidney damage in rats reared on a diet with a very low mineral content. It is probable that a deficiency of magnesium was the etiological factor concerned. Moore et al. (1936) noted that the main change in the kidneys was a marked injury to the interstitial and tubular tissue, although some fibrosis of Bowman's capsule and proliferation of the glomerular capillary endothelium was observed also.

9b. As noted above deficiency of magnesium results in the deposition of calcium in large amounts in the kidney. Cunningham and Cunningham (Greenberg 1939) observed the formation of renal calculi in rats receiving high calcium-low magnesium diets. Calculus

formation could be prevented by the addition of magnesium or phosphorus to the diet. The preventative effect of the addition of phosphorus was probably due to the precipitation of calcium phosphate in the intestine with a consequent reduction in absorption of calcium. However, Duckworth (1939) has reported the observations of Eveleth and Millen concerning a herd of sheep. On a diet high in magnesium and relatively low in calcium the herd was found to show a high incidence of renal calculi. This evidence would suggest that a disturbance of calcium-magnesium balance is associated with the production of renal calculus formation.

10. Effect on soft tissues. While the proportion of magnesium in bone, teeth and blood falls in animals on a diet deficient in magnesium there is no change in the magnesium content of soft tissues except in the liver where concentration of magnesium is increased (Watchorn and McCance 1937). Tufts and Greenberg (1938) observed, however, that the percentage of calcium increased in the heart, muscle, and particularly in the kidney. The increase was from 50 to 100 per cent in the heart and muscle and as much as fifteen-fold in the kidney. The most extensive studies of the effect of magnesium deprivation on calcium deposition in the soft tissues are those of Moore et al. (1936). They observed extensive calcification in the yellow elastic tissue of the endocardium and of the intima of the large arteries and veins. Similar deposits

were found on the surfaces of the diaphragm, the capsule of the spleen, and the Purkinje fibres. Calcification was also noted in muscle fibres. Therefore, one of the most important effects of magnesium deficiency appears to be a disturbance of calcium metabolism. Calcium seems to be mobilized from the skeleton and deposited in the soft tissues. It is remarkable that this should occur with such little change in the magnesium content of soft tissues. An explanation for the excess calcification may be found in the fact that bone salts form a magnesium reservoir in which magnesium and calcium are held in a fixed quantitative relationship. Reduction of the magnesium content will cause liberation of calcium. This, perhaps combined with the alteration in phosphatase activity due to the low serum magnesium, may furnish conditions suitable for the deposition of calcium salts in the soft tissues.

11a. Tetany due to deficiency. It is conceded by most investigators that it is difficult to raise calves on milk exclusively. Many of the calves develop tetany. Huffman et al. (1930) and Huffman and Duncan (1935, 1936) have demonstrated that this tetany is associated with a normal blood calcium but a low serum magnesium. They demonstrated that the addition of magnesium to the milk prevented the occurrence of tetany. Duckworth (1939) could not reconcile the signs of magnesium deficiency with the intake of magnesium. He stated that records of milk consumption revealed an intake of 160 grams which was

much in excess of the calculated requirement of 30 grams. However, Huffman et al. (1941) demonstrated that 12 to 15 milligrams of magnesium per kilo body weight would maintain normal serum magnesium levels only if supplied by alfalfa hay, corn, or corn gluten. When magnesium oxide was added to whole milk, 30 to 40 milligrams were required. It appears, therefore, that the form in which magnesium is administered is important. Also, the observation of McCance et al. (1942) that the amount of protein in the diet influences absorption of magnesium may play an important part in its assimilation.

11b. A condition known as "grass tetany" occurs in cows soon after they are turned out to pasture on fresh young grass in early spring (Maynard and Loosli 1943). The early symptoms are twitching of the muscles and nervousness. Later the animals may collapse in typical tetany. Blood magnesium values are always low, whereas calcium and phosphorus are normal. Concerning this finding of low plasma magnesium, Duncan et al. (1940) observed low values in normal cattle during May and June, which then increased until November and after that remained constant. It was their opinion that climate was an important factor in producing hypomagnesemia. However, the condition is cured by administration of adequate amounts of magnesium sulphate.

11c. Miller (1944) has described the occurrence of tetany in a 6 year old child who also had osteochondrosis of the capital

epiphysis of the femur. This tetany was associated with a normal plasma calcium and phosphorus and a low plasma concentration of magnesium (0.6 milligrams per 100 millilitres). This result was checked and the technique was considered accurate. The child, apparently on an adequate diet, was given 5 grams of magnesium sulphate three times a day for two weeks, at which time the plasma magnesium had risen to 2.6 milligrams per 100 millilitres and there was a concomitant decrease of irritability.

lld. It is apparent from the evidence presented above that in animals, at least, and in certain conditions in humans, tetany may be associated with a low serum magnesium and normal levels of calcium and phosphorus. Even when serum calcium is reduced, tetany does not develop if the magnesium concentration of the serum is increased (See page 33). It seems certain that tetany may be produced in more than one way and also that the situation in the tissues may not be reflected by the changes in the blood.

G. MAGNESIUM REQUIREMENTS OF MAN

1. Criteria. Since no clinical abnormality in man has been observed to be associated with a diet deficient in magnesium, the best standard available for determining magnesium requirement is that of Leitch (See page 21). This author has considered that an intake is adequate when it results in an equal number of cases of small positive and negative balances.

2. Adults. As stated on page 21 Tibbetts and Aub (1937) reported that hospital patients maintained small positive balances on an intake of 220 milligrams per day. Clark's data (Shohl 1939b) show that all of his prison subjects were in negative balance over a 28 week period although the intake was 250 milligrams. During the experiment these convicts all showed calcium retention which can be attributed to a previous calcium deficient diet. As it was likely they were in negative magnesium balance because they were excreting the extra magnesium which they had previously deposited in the bones in lieu of calcium, it seems reasonable to accept Tibbetts and Aub's figure.

3. Pregnancy. Information regarding the magnesium requirements of pregnant women is in fairly good agreement. Duckworth (1939) has stated that Coon et al. concluded that an intake of 350 to 450 milligrams daily was necessary during pregnancy. Hummel et al. (1937) found a retention of 60 milligrams on intakes of 350 to 430 milligrams daily. The magnesium requirements for foetal growth are slight up to the fourth month being 28 milligrams per day. Thereafter, the demands are greater until at birth the magnesium content of the foetus is estimated between 277 and 780 milligrams (Givens and Macy 1933).

4. Children. Daniels (1941) observed negative to slightly positive balances in pre-school children who were ingesting 10.4 to 11.4 milligrams per kilo. There was regular retention of magnesium

when intakes were increased above 11.8 milligrams per kilo.

5. Average intake. The availability of magnesium in certain elective American diets has been reported by Tigerstedt (Schmidt and Greenberg 1935). The results are summarized in Table V. From these figures it is evident that with the usual intake of 2000 to 2500 calories there is an intake of magnesium which is in excess of the amount claimed to be adequate by Tibbetts and Aub. Despite the lack of clinical evidence concerning deficiencies of magnesium, Duckworth (1939) has pointed out that it is difficult to decide to what extent this may occur in humans.

H. FACTORS AFFECTING THE CONCENTRATION OF MAGNESIUM IN THE BLOOD

1. Introduction. The mechanism by which the concentration of magnesium in the blood is maintained at a relatively constant level is not known. However, various conditions have been reported which are associated with alterations in the magnesium content of serum. In general the reports are concerned mainly with variations in total serum magnesium although it is possible that measurements of the non-diffusible portion of serum magnesium would be more revealing. The evidence available regarding alterations of non-diffusible magnesium is concerned mainly with disorders of the thyroid gland and because this evidence is particularly pertinent to the present study it will be considered in a separate section.

TABLE V

DAILY INTAKE OF MgO IN ELECTIVE DIETS OF U.S.A.

Calories	MgO expressed in milligrams
> 4000	890
3500 - 4000	510
3000 - 3500	500
2500 - 3000	460
2000 - 2500	320
1500 - 2000	230

2a. Endocrine. Although Bulger and Gausmann (1933) found no increase in serum magnesium following the administration of parathyroid hormone, many workers have shown that slight increased do occur (Melli and Konradinova, quoted by Duckworth 1939, Greenberg and Mackay 1932a, Scholtz 1931). Greenberg and Mackay (1932a) demonstrated that the increase in magnesium concentration occurred considerably before the increase in calcium manifested itself, taking place between the second and sixth hours after the injection and then quickly dropping back to initial levels. The increase in magnesium was relatively small being on the average 0.6 milligrams per 100 millilitres of plasma. Denis and Talbot (1921) have reported low serum magnesium values in some human cases of parathyroid deficiency. This has been confirmed by Hirschfelder and Haury (1934), Bulger and Gausmann (1933) and Hartog and Muller (Haury 1942). A few cases of hyperparathyroidism reported by Tibbetts and Aub (1937) showed a relatively normal serum magnesium in spite of a hypercalcaemia.

2b. Duckworth (1939) has reported that Carnavo found increases in blood magnesium following injections of anterior pituitary extracts into dogs and rabbits. But Borgstrom (1938) did not observe this effect. However, it is possible the difference in these results could be explained by the variability in potency of the pituitary extracts used. Injection of prolan has no effect on

blood magnesium in man (Duckworth 1939). Furthermore, no change in blood magnesium has been found to occur in cases of pituitary dysfunction (Blumgarten and Rohdenberg 1927). Gerschman (1943) has reported recently that while hypophysectomy or removal of the pars distalis decreased all plasma minerals, magnesium was affected to the greatest extent.

2c. Variations in human serum magnesium in connection with menstrual irregularities were recorded by Watchorn (1926). This author claimed that there was a premenstrual decrease in serum magnesium concentration in patients who had general malaise but no pain prior to the onset of menstruation. However, an analysis of the evidence reveals that out of 14 subjects, 5 showed no change, 3 had an increase and only six had a decrease in serum magnesium. Obviously this is insufficient evidence on which to base conclusions.

2d. It has been reported that blood magnesium fluctuates widely in pregnancy (Duckworth 1939). The maximum concentration occurs between the fifth and seventh months and subnormal values at term. McCance and Watchorn (1931) also found low values in cases between the thirty-second and thirty-eighth week of gestation. In contrast to this it has been found that in the cow, serum magnesium tended to rise at parturition (Allcroft and Godden 1934, Godden and Duckworth 1935).

3. Deficiency and administration of magnesium. In experimental magnesium deficiency both red cell and plasma magnesium are lowered (See page 44). The diffusible and non-diffusible fractions of plasma magnesium are both decreased so that the percentage of diffusible magnesium remains approximately the same (Hoobler et al. 1937). When magnesium salts are injected subcutaneously the increase of plasma magnesium reaches its maximum in 2 hours (Haury and Cantarow 1940, Whelan 1925). As would be expected intravenous injection results in an immediate rise in serum magnesium which then gradually falls to normal levels in 5 to 6 hours (Denis 1923). Magnesium chloride administered by mouth to dogs has been shown to cause a rise in serum magnesium (Winter and Richey 1931). The oral administration of magnesium has very little, if any, effect on the concentration of magnesium in plasma of humans but apparently can be absorbed by sheep and result in an increase in the serum magnesium (Duckworth 1939).

4. Effect of administration of other salts. The injection of calcium salts also produces an increase in the amount of plasma magnesium, the peak coming about 3 hours after the injection (Schmidt and Greenberg 1935). The magnesium level then subsides. Shohl (1939c) has stated that phosphate injections, not sufficient to cause a change in calcium, result in a decrease in plasma magnesium. In conditions such as oxalate poisoning which cause a

depression of serum calcium, the serum magnesium has been found to be elevated (Jacoby and Friedel 1933). Injections of sodium sulphate and of sodium chloride appear to have no effect on serum magnesium (Brookfield 1934).

5. Inanition. Schmidt and Greenberg (1935) state that a continuous supply of food is not essential to maintain the serum concentration of magnesium. During long periods of starvation in animals the magnesium level of serum is maintained. Thus no decrease in serum content of magnesium occurred in starving dogs and cats until they have had a 20 per cent loss in weight. It seems likely that the maintenance of serum magnesium depends on the release of magnesium from the tissues, particularly the skeleton.

6. Effect of temperature. Recently, it has been reported that hypothermia produced in rabbits by experimental methods is associated with a definite rise in serum magnesium (Steadman et al. 1943). This finding is of particular interest in view of the observations made by Suomalainen (1938) that in the hypothermic state of hibernation a similar increase occurred. He has demonstrated also that there was a rise in serum magnesium in normal human beings during sleep. The significance of these results and their possible association with thyroid activity is discussed in a later section (See page 91). Duncan et al. (1940) who studied the plasma magnesium

of normal cattle for a period of a year, observed uniformly low values during May and June. These values increased until November and remained constant during the winter. They stated that climatic and other non-dietary factors were concerned but it seems possible that the eating of grass from spring pasture during May and June might be a cause in lieu of the studies of "grass tetany" (See page 50). With respect to the effect of injection of magnesium salts on body temperature it has been reported by Taylor and Winter (1929) that in feverish dogs temperature changes were found proportional to changes in the blood magnesium and not related to the dose of the salt. Each increase of 2 milligrams per 100 millilitres of serum gave a reduction in temperature of about 1 degree Fahrenheit.

7. Renal disorders. Although Denis and Hobson (1923) reported normal magnesium values in a series of 19 nephritic patients, more recent evidence has accumulated which appears to indicate that retention of magnesium occurs in cases of renal insufficiency. It was demonstrated by Bechner and Hamann that patients with diseased kidneys and normal renal function had normal serum magnesium but if renal function was impaired serum magnesium was elevated (Haury 1942). Watchorn and McCance (1932) have observed the serum magnesium to range from 3.07 to 10.34 milligrams per 100 millilitres in eight cases of uremia. Hirschfelder and Haury (1934) demonstrated that in animals with injured kidneys the injection of magnesium produced a

rapid increase in the concentration of magnesium on the serum.

Haury (1942) has stated that Raices, Hartog and Muller, and Lucchi have confirmed these findings, as have Walker and Walker (1936), Brookfield (1937) and Bernstein and Simkins (1940).

8. Hypertension. A slight increase in serum magnesium has been reported in patients with hypertension not associated with kidney disease (Wacker and Fahrig 1932, Walker and Walker 1936, Bernstein and Simkins 1940). However, the increases are within the range of presently accepted normal levels and therefore, little significance can be attached to them.

9. Asthma. The fact that intravenous injections of magnesium will relieve the spasm of the bronchi in clinical cases of asthma (Haury 1940), and that magnesium acts to dilate the bronchi when perfused through excised guinea-pig lungs (Haury 1938) has led to the investigation of the concentration of magnesium in the serum of patients with asthma. Haury (1940) has found that 50 per cent of patients examined during an acute attack of bronchial asthma had a low serum magnesium.

10. Epilepsy. Since it has been demonstrated that a deficiency of magnesium may lead to convulsions it has been considered that a deficiency in plasma magnesium might be associated with epileptic convulsions. Low plasma magnesium has been found to be associated

with epilepsy by Denis and Talbot (1921), Blumgarten and Rohdenberg (1927), and Wolf (1936). However, in twenty-two cases studied by Greenberg and Aird (1938) the blood serum magnesium was essentially normal.

11. Cancer. A great many attempts have been made to associate magnesium with the growth, distribution and production of cancer. None of the evidence is convincing however, and the present conception is that no association exists. Shear (1933) has collected an excellent bibliography of the literature up to 1933. Further references may be found in reports by Sugiuria and Benedict (1935) and Haury (1942).

12. Diabetes. Information concerning the concentration of magnesium in this disease is meagre. Brookfield (1937) and Watchorn and McCance (1932) reported three patients in diabetic coma having a high magnesium. However, a number of cases not in coma had a normal magnesium level. In diabetic patients not in coma, Blumgarten and Rohdenberg (1927) found three out of seven cases to have a high serum magnesium.

I. RELATION OF MAGNESIUM METABOLISM TO THYROID DISEASE

1. Introduction. Following the previous discussion of the specific roles played by magnesium in the life processes of the

animal organism, it is now possible to approach the subject of association between thyroid disorders and magnesium metabolism with a better understanding. In the introductory chapter, mention was made of some evidence which indicated that the metabolism of magnesium may be associated with the function of the thyroid gland. In particular, the evidence concerning variations in the amounts of diffusible magnesium of plasma associated with disorders of thyroid function is the most pertinent. Accordingly, the reports concerning these variations require detailed review.

2a. Relationship found by Soffer et al. Previous investigations have determined that magnesium exists in human plasma in diffusible and non-diffusible forms (See page 15). The non-diffusible form is presumed to be bound to protein of the plasma. In 1939 Soffer et al. first reported that the amount of this non-diffusible magnesium was significantly higher in hyperthyroidism than in normal individuals. They gave the term "bound" magnesium to that portion of total serum magnesium which was not diffusible through a cellophane membrane. Soffer and his associates (1939) studied fourteen normal individuals and in 1941 expanded their group of patients to fifty. The bound magnesium of normal persons varied from 3.1% to 22.1% of total plasma magnesium whereas in the fifty hyperthyroid patients, fifteen had bound magnesium levels which fell within normal limits (under 25%) and thirty-five showed bound magnesium levels above

normal. They also studied seven patients with myxedema, five of which had no bound magnesium, the remaining two showing small amounts of 5.7% and 2.8% respectively.

2b. These authors also reported that bound magnesium increased to normal levels in myxedematous patients treated with thyroid extract. Conversely, in hyperthyroid individuals the bound portion of serum magnesium fell to normal values following thyroidectomy. They confirmed these results experimentally by using dogs, whose thyroid glands had been removed. They showed that bound magnesium levels fell after thyroidectomy and increased when thyroxine was administered (Soffer et al. 1941). The method used for magnesium determinations was that of Briggs (1922). The total serum magnesium was separated into diffusible and non-diffusible fraction by ultrafiltration of serum through a No. 600 cellophane membrane under a pressure of 80 pounds of nitrogen per square inch but the apparatus for doing this was not described.

3. Contrary results of Cope and Wolff. The above results seemed to indicate that there was some relation between thyroid function and magnesium metabolism. However, Cope and Wolff (1942) could not confirm these observations. In a series of seventeen normal individuals they found the bound magnesium to average 37.0% (range 14-56) of the total magnesium and in twenty-four patients with hyperthyroidism this bound fraction to average 35.0%. These authors used a

modification of the Briggs method for estimating magnesium. To obtain the non-diffusible portion, serum was subjected to a constant air pressure of approximately 40 pounds per square inch and forced through a No. 300 cellophane membrane. The thickness of the membrane did not account for the difference in results as similar values were obtained when a No. 600 cellophane was used.

4. Confirmation of Soffer et al. A confirmation of Soffer's work was published by Dine and Lavietes (1942). These authors used a type of apparatus in which the diffusible portion of serum magnesium was obtained under rigid anaerobic conditions. In their fourteen normal subjects, bound magnesium was 14 - 31% of total serum magnesium and in nine patients with hyperthyroidism bound magnesium exceeded 31%. In four individuals with myxedema the bound portion of serum magnesium was nil. Their bound magnesium values for normal persons were significantly higher and their estimations of total serum magnesium were lower than those of Soffer et al. They suggested that the difference in total serum magnesium values may have been due to differences in technique since they used an ashing procedure instead of precipitating the plasma proteins with trichloroacetic acid.

5. Contrary results of Bissell. Bissell (1945) using the same anaerobic apparatus for obtaining ultrafiltrates and similar technique for measurement of magnesium could not confirm the results of Dine and Lavietes. In twenty-four hyperthyroid patients he obtained a mean

value of 30.5 S.D. \pm 9.1% (range 17-42) for the bound magnesium. In eighteen normal individuals bound magnesium values averaged 30.4 S.D. \pm 1.1%.

6. Summary. The results obtained by these various investigators have been summarized in Table VI. It is to be noted that there is no marked difference of the total serum magnesium of hyperthyroid, hypothyroid or normal individuals. All these authors used the method of Briggs or some modification in estimating magnesium in serum and ultrafiltrates. In determining total serum magnesium Dine and Lavietes used an ashing procedure which they suggest may account for their lower total magnesium values. Various techniques of ultrafiltration were used. While Soffer used 80 pounds Nitrogen pressure per square inch, Cope and Wolff used 40 pounds air pressure per square inch. These authors obtained opposite results. Both Dine and Lavietes and Bissell used the same type of anaerobic ultrafiltration apparatus but their results do not agree. Evidently then, there are inexplicable conflicts in the reports concerning the alteration in the amounts of bound magnesium in disorders of the thyroid gland.

J. SUMMARY OF REVIEW OF LITERATURE.

1. Methods. In this review an attempt has been made to bring together the most important work on magnesium metabolism and the various functions of magnesium in the animal organism. The different

TABLE VI

EFFECTS OF DYSTHYROIDISM ON SERUM MAGNESIUM REPORTED BY DIFFERENT AUTHORS

Name of Authors	Total serum magnesium in mgm. per cent		Bound magnesium expressed as percentage of total serum magnesium	
	Normal	Hyper-thyroidism	Normal	Hyper-thyroidism
Soffer et al. (1939, 1941)	2.52	2.44	14.5	36
Cope & Wolff (1942)	1.99	1.88	14 - 56	35
Dine & Lavietes (1942)	1.6-2.2	1.4 - 2.0	14 - 31	31 - 50
Bissell (1945)	2.28	1.87	17 - 42	30.5
				Myxedema
				1.2
				0

methods of estimating magnesium have been discussed and it has been pointed out that the new titan yellow methods show a marked improvement over the older techniques.

2. Occurrence and distribution of magnesium. Magnesium occurs in various foodstuffs and particularly in chlorophyll. Therefore green vegetables are an important source of the mineral. In the animal organism it occurs in all tissues of the body. The skeleton has the highest concentration but unlike calcium it only contains about one half of the body magnesium. Magnesium is then an important constituent of the soft tissues of the body. In normal serum it is present in amounts which range from 2 to 3 milligrams per 100 millilitres. The content of blood cells is about twice this value. In plasma, magnesium is present in two forms, diffusible and non-diffusible. About 70 per cent of magnesium in plasma is diffusible. The parathyroid gland exerts some influence over magnesium but not to the same extent as it does over calcium. A decrease in body temperature is accompanied by a fall in concentration of the serum magnesium.

3. Metabolism of magnesium. A study of the metabolism of magnesium reveals that this element is absorbed and excreted by the animal organism. Extra magnesium intake is excreted mainly by the intestine as is the case with calcium. This excessive intake of

magnesium produces a negative calcium balance in the body. Little difference in magnesium balance from the normal occurs in hyperparathyroidism, exophthalmic goitre, Cushing's disease or other metabolic disorders. This may not be the case, however, if studies are carried out in which the physiologically active part (diffusible magnesium) of plasma magnesium is estimated. Very little is known regarding the interrelationship of vitamins and magnesium metabolism but it has been demonstrated that magnesium acts as an important activator of many enzyme systems and is a constituent of carboxylase.

4. Pharmacology of magnesium. The evidence is insufficient to show that poisoning by magnesium can occur by absorption from the intestine except possibly in patients with renal insufficiency. Injections of magnesium sulphate in sufficient dosage cause a slowing of the heart and a depression of the nervous system both centrally and at the neuromuscular junction. Magnesium sulphate may act as a cholagogue but other agents are more effective. Intravenous injections of magnesium seem to result in a hyperglycaemia and glycosuria. Hyaline casts may appear in the urine and the temperature of the body may be depressed.

5. Deficiency and requirements. The most obvious effect of both deficiency and excess of magnesium is an upset in calcium metabolism shown by disturbance of bone calcification and by deposition of

calcium in soft tissues. Low concentration of magnesium in the plasma can be associated with signs of tetany even if the serum calcium level is normal. The daily requirement of magnesium appears to be approximately 200 to 300 milligrams per day for an adult. This is supplied by the average diet. Therefore clinical states of deficiency are not likely to occur in man unless absorption or excretion of magnesium is disturbed.

6. Serum levels in disease. The serum magnesium is raised in patients with renal insufficiency. Variations have been reported in other diseases but in most of these the changes are insignificant. Disorders of the thyroid gland are an outstanding exception and it is the purpose of this thesis to help resolve the conflict on this point.

CHAPTER III

METHOD

A. METHOD OF THE DETERMINATION OF MAGNESIUM

1. Preparation of the plasma. Venous blood was withdrawn from patients in the post-absorptive state using heparin as an anticoagulant. The blood was centrifuged at 2500 revolutions per minute for 10 minutes and plasma pipetted off and stored in a refrigerator at approximately 5° Centigrade. It was found that no appreciable change in the concentration of magnesium occurred even if the plasma was stored as long as a month. A 2 millilitre aliquot of plasma was used for total plasma magnesium determinations. To obtain the diffusible fraction 3 millilitres were subjected to ultrafiltration. The value for the non-diffusible or "bound" magnesium was obtained by subtracting the diffusible fraction from the total.

2. Procedure. The titan yellow method of Kunkel et al. (1947) was used to measure magnesium. To 2 millilitres of plasma were added 4 millilitres of distilled water, 2 millilitres of 10% sodium tungstate followed by 2 millilitres of 0.67 normal H₂SO₄. This was mixed thoroughly and filtered, using a Whatman No. 41 filter paper.

If the filtrate was not clear it was refiltered. Three millilitres of the filtrate were transferred to a 10 millilitre volumetric flask. One drop of 0.01% methyl red was added to act as an indicator and the filtrate was carefully neutralized with a 0.1% NaOH solution. One millilitre of a 2% solution of hydroxylamine hydrochloride, 1 millilitre of a 0.075% aqueous solution of titan yellow, and 2 millilitres of 6.0% NaOH solution were added in succession followed immediately by distilled water to bring the volume to 10 millilitres. The solution was then transferred to colorimeter tubes and placed in a water bath at 25° Centigrade for 10 minutes. The percentage transmission was read by the use of the Evelyn colorimeter at a wave length of 540 millimicrons.

3. Standard solution. The standard solution of magnesium was made up using magnesium filings rather than $MgSO_4 \cdot 7H_2O$ which was used by Kunkel et al. (1947). By doing this it was thought that any error which might occur due to the deliquescent properties of the magnesium sulphate heptyhydrate would be avoided. Nineteen milligrams of the magnesium filings were dissolved in 0.5 millilitres of concentrated HCl and the solution made up to 1 litre by adding distilled water. One millilitre of this solution contained 19 micrograms of magnesium.

4. Calibration Curve. The magnesium standard solution was used

in preparing the calibration curve. A series of 10 millilitre volumetric flasks was set up containing the following amounts of magnesium; 0.0 (blank), 4.75, 9.5, 19, 28.5, 38 micrograms. These standards were treated exactly as outlined above for plasma. The transmittance was plotted along the ordinate and the amounts of magnesium along the abscissa of the graph (See Fig.1). It was found that the calibration curve changed its slope from day to day. This was due to some change in the concentration of color in the titan yellow solution. This solution had to be kept in the refrigerator and was made up fresh every 10 days. To overcome any error due to this change, however, a calibration curve for the standard solution was determined daily.

5a. Accuracy of the method. To determine whether results could be reproduced using this method, eight separate magnesium determinations were made on 2 millilitre aliquots of the same plasma. For these eight determinations, a mean of 2.48 milligrams per cent was obtained with a range of 2.41 to 2.58 and a standard deviation of $\pm 0.05\%$. In recovery experiments, known quantities of magnesium were added to 2 millilitre aliquots of plasma. Magnesium determinations on ten samples gave results as recorded in Table VII. It appears, therefore, that the titan yellow method affords an accurate means of estimating magnesium in human plasma.

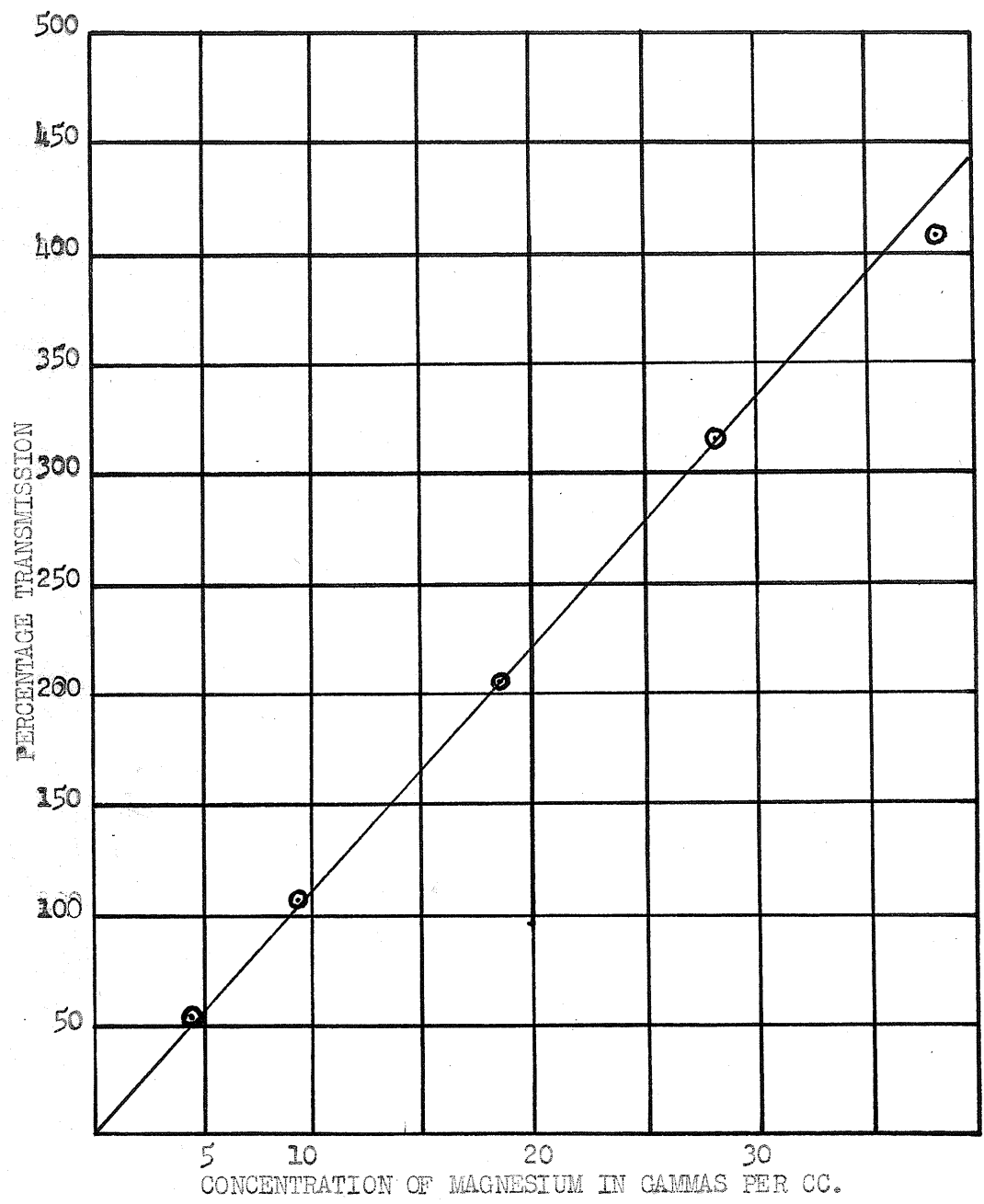


FIGURE 1. CALIBRATION CURVE FOR STANDARD MAGNESIUM SOLUTION.

TABLE VII. PERCENTAGE OF ADDED MAGNESIUM RECOVERED FROM 2 cc.

ALIQUOTS OF PLASMA.

Sample	Total Magnesium per 2 cc. of Plasma in ugm.	Added Magnesium in ugm.	Obtained Results Magnesium in ugm.	Amount of Magnesium Recovered in ugm.	% of Added Magnesium Recovered
1	14.5	19	33.3	18.8	99
2	16.5	19	36.6	20.1	106
3	15.0	19	33.3	18.3	97
4	14.3	19	33.1	17.8	99
5	16.5	19	36.5	20.0	105
6	14.5	19	33.0	18.5	97
7	15.3	19	35.0	19.7	104
8	14.5	19	33.0	18.5	98
9	15.3	19	33.5	18.2	96
10	14.5	19	33.3	18.8	99

5b. Other factors. In expressing the results no allowance has been made for the space occupied by plasma proteins because Watchorn and McCance (1932) have reported that although there seems to be a tendency for the diffusible magnesium to be inversely proportional to the total serum protein, the relationship is not sufficiently significant to be important. Justification for this omission was also obtained by analogy with the behaviour of calcium. Smith and Sternberger (1932) investigated the relationship of diffusible calcium to serum proteins and stated that a 50 per cent variation in serum protein concentration would cause only a 2.5 per cent error in the result for the diffusible fraction. Furthermore, no effort was made to control the pH of the serum. Again considering the analogous element, calcium, it has been shown by Greenberg and Gunther (1929) that within the range of pH 7 to 8 there was no marked influence of hydrogen ion concentration on the distribution of diffusible and non-diffusible calcium fractions. It was found on nine samples that the method by which the plasma was prepared in this study did not extend the hydrogen ion concentration beyond this range.

B. ULTRAFILTRATION APPARATUS

1. Description. To obtain the diffusible portion of plasma an apparatus similar to that described by Nicholas (1932) was designed

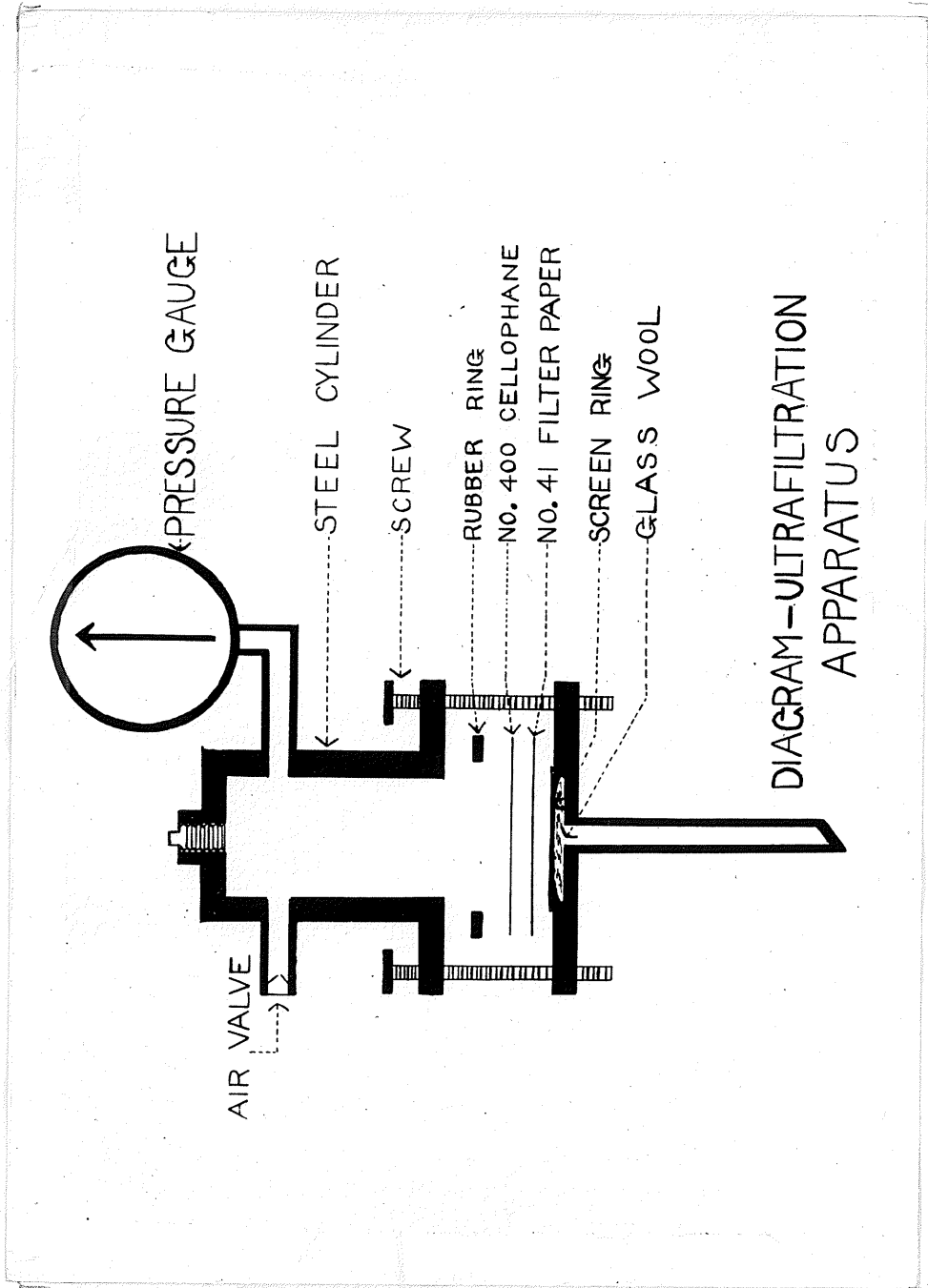


Figure 2. Ultrafiltration Apparatus

whereby plasma could be subjected to increased air pressure and forced through a No. 300 cellophane membrane. The apparatus is illustrated in Figure 2. The diagram is in an exploded form to illustrate the parts more clearly. In assembling the apparatus, glass wool was placed on the funnel portion. Then in the following succession, screen ring, filter paper, cellophane, and rubber ring were laid one on top of the other. Finally, the bottom funnel part was screwed tightly to the upper steel cylinder. Plasma was introduced through in inlet at the top and the inlet was made air tight by a screw dipped in wax. Air under pressure was then introduced until the pressure gauge indicated 125 pounds pressure per square inch. By placing the whole apparatus in an automatic shaker, 1 millilitre of ultrafiltrate was obtained in approximately 1 hour. All the ultrafiltrations were carried out at room temperature which varied from 21° to 28° Centigrade.

6b. Experiments to test performance. The performance of the filter was checked by testing all filtrates for protein with trichloroacetic acid. In addition, several experiments were carried out to ascertain the factors which might affect the magnesium content of the ultrafiltrate and to find the optimum conditions for operation of the apparatus. The concentration of magnesium in the ultrafiltrate was found not to be affected by varying the air pressure (See Table VIII), or by using glass wool, soaking the cellophane in

TABLE VIII

EFFECT OF CHANGE OF PRESSURE ON SPEED OF ULTRAFILTRATION

Pressure in lbs. per square inch	Concentration of magnesium in ultrafiltrate in mgm.%	Time taken to obtain 1 cc. of ultrafiltrate
125	1.10	55 min.
	1.15	60 "
	1.09	60 "
100	1.18	85 min.
	1.10	90 "
	1.15	80 "
80	1.12	185 min.
	1.11	175 "
	1.06	185 "
50	1.15	200 min.
	1.10	200 "
	1.18	195 "

distilled water for varying periods, using or not using the automatic shaker. (See Table IX)

6c. Reproducibility of results. The reliability of the results was ascertained by showing that a second passage of an ultrafiltrate did not alter its concentration of magnesium. Similarly, distilled water did not gain magnesium when passed through it. Seventeen different samples of the same plasma were passed through this apparatus and magnesium determinations of the ultrafiltrates from these samples gave a mean of 1.29 milligrams per cent with a range of 1.18 to 1.38 and a standard deviation of $\pm 0.05\%$. It was evident, therefore, that a reasonable accuracy could be obtained by the use of this apparatus.

C. SELECTION OF SUBJECTS.

1. Normal control group. This group consisted of 6 males and 6 females who were laboratory technicians or medical students going about their daily work. Their ages ranged from 20 to 30 years.
2. Dysthyroid group. All patients with disorders of the thyroid were chosen as typical clinical examples of myxedema, hyperthyroidism and nodular non-toxic goitre. Clinical diagnosis was confirmed by basal metabolic readings and protein bound iodine determinations. With the exception of one in the hyperthyroid group, all patients were either on the wards or were out patients of the Winnipeg General

TABLE XIX

RESULTS CONCERNING FACTORS AFFECTING SPEED OF ULTRAFILTRATION PROCESS*

Conditions	Concentration of magnesium in ultrafiltrate in mgm.%	Time taken to obtain 1 cc. of ultrafiltrate
With Glass Wool	1.47	170 min.
	1.36	185 "
	1.40	175 "
Without Glass Wool	1.47	425 "
	1.43	440 "
	1.32	430 "
Cellophane soaked 30 days and Glass Wool used	1.31	180 min.
	1.28	165 "
	1.24	190 "
Cellophane soaked 4 hours and Glass Wool used	1.23	270 "
	1.30	260 "
	1.25	275 "
With shaking, Cellophane soaked 30 days and Glass Wool used	1.31	60 min.
	1.28	65 "
	1.25	60 "
Without shaking, Cellophane soaked 30 days and Glass Wool used	1.37	185 "
	1.29	175 "
	1.32	180 "
Shaking, Cellophane soaked 30 days, Glass Wool used and 6 cc. of plasma in ultrafilter	1.25	55 min.
	1.27	60 "
	1.35	60 "
Same conditions except 3 cc. of plasma in ultrafilter	1.28	65 "
	1.37	60 "
	1.30	65 "

* An air pressure of 125 lbs. per sq. inch was used for all experiments

Hospital. The clinical data of these patients are recorded in Tables X, XI, XII.

3. Miscellaneous group. In this group were 5 patients with psychoneurosis and single examples of Paget's disease, cirrhosis of the liver, psychosis, infectious hepatitis, congestive heart failure, essential hypertension, carcinoma of the pancreas with jaundice, myelogenous leukemia and diabetes mellitus. The clinical data of these patients are recorded in Table XIII.

TABLE X. CLINICAL DATA ON PATIENTS WITH TOXIC GOITRE

Case No.	Sex	Age	B.M.R.	Weight	Temp.	Pulse	Protein Bound Iodine μ gm. %	Cholesterol mgm. %	Total Protein gm. %	Clinical Notes
A4051	F	26	+ 78	122	98.8	104	19.7	105	6.05	Loss of 50 lbs.- 3 years, excessive appetite, irritable, amenorrhea and sterility, exophthalmos, hot moist skin, tremor of hands and tongue, diffuse enlargement of thyroid gland. Diagnosis: Diffuse toxic goitre with exophthalmos.
16051	F	30	+ 57	86	98.8	148	17.2	178	7.4	Difficulty swallowing 1 month, irregularity of menstruation, always hungry, nervous, excessive sweating, palpitation, no exophthalmos, adenoma, rt. lobe of thyroid, tremor of hands, Diagnosis: Toxic adenoma of thyroid.
16383	F	37	+ 33	138	98.2	100	15.0	228	6.4	Thyroidectomy 6 months ago. Signs and symptoms have recurred. Nodules in right lobe. Diagnosis: Recurrent nodular toxic goitre with exophthalmos.
15562	F	59	+ 42	105	98.6	100	19.1	-	7.0	Weakness 5 months. Rapid weight loss, palpitation, warm skin, no exophthalmos. Substernal thyroid. B.P. 174/88. Diagnosis: Toxic adenoma of thyroid.
16469	M	63	+ 62	101	98.0	90	14.0	-	6.6	Diabetes mellitus. Skin hot. Exophthalmos, tremor, diffusely enlarged thyroid. Diagnosis: Diffuse toxic goitre with exophthalmos, Diabetes mellitus.
6900	M	62	+ 53	133	98.0	72	15.9	217	7.3	Nervousness, heat intolerance, weight loss, profuse sweating, tremor. B.P. 155/85. Diagnosis: Nodular toxic goitre.
A5491	F	55	+ 52	114	98.0	96	23.0	228	6.5	Irritable and nervous. Weakness 2 years. Good appetite. Weight loss. No exophthalmos, B.P. 150/75. Diagnosis: Diffuse toxic goitre.
A6017	F	37	+ 29	90	98.0	90	12.8	160	6.1	Amenorrhea. Rapid weight loss, palpitation, appetite good, skin hot, tremor of hands. Thyroid diffusely enlarged. B.P. 144/70. Diagnosis: Diffuse toxic goitre.
16469	F	34	-	-	-	100	20.0	-	6.5	Loss of weight. Palpitation, dyspnea. Exophthalmos. Hard, diffusely enlarged thyroid. Auricular fibrillation. B.P. 150/90. Diagnosis: Diffuse toxic goitre with exophthalmos and congestive heart failure.

TABLE XI. CLINICAL DATA ON PATIENTS WITH HYPOTHYROIDISM.

Case No.	Sex	Age	B.M.R.	Weight	Temp.	Pulse	Protein Bound Iodine μ gm. %	Cholesterol mgm. %	Total Protein gm. %	Clinical Notes
15166	F	52	- 35	145	96.4	56	1.1	379	7.6	Weakness. Increase in weight. Swollen hands and feet. Dry skin. Sensitive to cold. B.P. 100/70. Diagnosis: Primary myxedema.
13555	F	67	- 24	120	97.6	60	1.0	295	7.3	Easily fatigued. Weakness. Skin dry and scaly. Eyelids edematous. Hands and feet swollen. B.P. 170/100. Diagnosis: Primary myxedema.
16004	F	62	- 26	130	98.2	64	2.6	-	7.1	Extreme lassitude. Weakness. Constipation. Increase in weight. Skin dry. Heart enlarged to left. B.P. 140/100. Diagnosis: Primary myxedema.
16306	M	72	- 30	171	96.4	52	1.5	311	7.4	Diagnosed myxedema 15 years ago. Stopped thyroid. Signs and symptoms have recurred. Heart enlarged to left. B.P. 170/80. Diagnosis: Primary myxedema, diabetes mellitus.
16205	M	35	- 40	156	98.6	56	2.0	342	6.5	Extreme weakness. Sensitive to cold. Skin dry. Voice husky. Low QRS in leads 1,2,3 of E.K.G. Diagnosis: Primary Myxedema.
A1075	F	41	- 47	133	96.8	72	1.4	-	6.4	Menopausal. Thyroidectomy 1933. Mental depression with hallucinations. Eyebrows sparse. Skin dry. B.P. 140/100. Diagnosis: Secondary myxedema.
A486	F	65	- 23	154	98.0	56	3.7	290	6.5	Tires easily. Increase in weight. Skin dry. Eyelids and hands edematous. B.P. 140/90. Diagnosis: Primary myxedema.
A345	F	70	- 30	151	97.2	84	1.0	267	6.5	Diagnosed myxedema 1944. Off thyroid 1 month. Signs and symptoms have recurred. Heart enlarged to left. B.P. 210/120. Diagnosis: Primary myxedema, essential hypertension.
13592	M	44	- 45	140	96.8	48	-	347	7.8	Progressive weakness. Falling hair. Voice husky. Skin dry. Peri-orbital edema. B.P. 140/90. Diagnosis: Primary myxedema.
10618	F	18	- 47	107	97.6	68	0.0	260	-	Loss of energy. Dull at school. Skin dry. Sensitive to cold. Typical myxedematous facies. Speech slow. B.P. 100/70. Diagnosis: Juvenile myxedema.

TABLE XII. CLINICAL DATA ON PATIENTS WITH NON TOXIC GOITRE

Case No.	Sex	Age	B.M.R.	Weight	Temp.	Pulse	Protein Bound Iodine $\mu\text{gm.}\%$	Cholesterol $\text{mgm.}\%$	Total Proteins $\text{gm.}\%$	Clinical Notes
15420	F	37	+ 3	112	98.8	88	4.8	225	7.6	Nervous breakdown 3 years ago. Now complains of headaches and nervousness, fainting spells, palpitation. No eye signs. Hair and skin normal. Coarse tremor of hands. Nodule in left lobe of thyroid 3 inches in diameter. B.P. 120/76. Diagnosis: Non toxic adenoma, anxiety state.
64344	F	33	+ 7	163	98	68	7.3	200	7.1	Parasthesias both arms. Nervousness. Hot flushes and dyspnoea when excited, headaches. Appetite poor. Sleeps well. Menstrual cycle regular. Skin normal. No tremor. Diffusely enlarged thyroid. B.P. 136/90. Diagnosis: Non toxic diffuse goitre, hypochondriasis.
15814	F	30	+ 21	117	98.6	56	5.5	168	6.7	Noticed neck becoming larger. Came to O.P.D. for advice. No loss of weight. Appetite fair. No temperature. No eye signs. No tremor. Skin normal. Round nodule, 1 cm. in diameter opposite cricoid cartilage. Pulse 56 / minute. B.P. 132/90. Diagnosis: Non toxic nodular goitre.
15865	F	52	+ 2	169	98.2	80	5.2	187	7.0	Headache, earache, red nose, indigestion. No other complaints. Nodular goitre discovered on physical examination. No eye signs. Obese. Skin normal. No tachycardia. Acne rosacea of nose. B.P. 160/90. Diagnosis: Non toxic nodular goitre, acne rosacea.
5539	F	64	- 8	142	97.6	72	5.1	210	6.7	No complaints. Nodular goitre discovered during routine examination. Right lobe of thyroid enlarged and nodular. No eye signs. Skin normal. No weight loss. B.P. 162/92. Diagnosis: Non toxic nodular goitre.
A 981	F	79	- 6	110	98.2	112	5.6	137	7.2	Dysphagia. Chronic cough. Goitre for 20 years. Thin. No eye signs. Nodule size of orange left lobe of thyroid. Deviation of trachea. Skin normal. Basal rhonchi both lungs. B.P. 150/90. Diagnosis: Non toxic nodular goitre, chronic bronchitis.

TABLE XIII. CLINICAL DATA ON PATIENTS WITH MISCELLANEOUS DISEASES.

Case No.	Sex	Age	B.M.R.	Weight	Temp.	Pulse	Protein Bound Iodine ugm. %	Cholesterol mgm. %	Total Proteins gm. %	CLINICAL NOTES
10818	M	66	-	-	-	-	6.0	153	7.2	Dyspnoea on exertion 2 years, marked oedema, fibrillation, enlarged heart. Diagnosis: Rheumatic heart disease and congestive failure.
15714	F	64	+ 43	99	98.6	88	9.6	-	6.2	Dyspnoea for years, thyroidectomy 16 and 8 years ago. Signs of advanced failure. B.P. 160/60. Diagnosis: Hypertensive heart failure.
15385	M	71	- 0	143	98.4	80	8.0	-	6.6	Mentally confused, pain in the back, febrile urethral structure, pyuria. Diagnosis: Pyelitis and Paget's disease.
Child C.	F	6	-	-	-	-	6.6	-	7.1	Mother unstable, child difficult to control, no abnormal physical findings. Diagnosis: Behaviour problem.
9604	M	63	+ 63	131	98.0	68	3.9	213	7.3	Violent confused, inaccessible. Diagnosis: Involutional depression. Physical examination essentially negative.
47631	F	49	- 5	184	97.6	68	4.4	-	6.9	Dyspnoea, headaches and palpitation. B.P. 240/150. No signs of congestive failure. Diagnosis: Essential hypertension.
63593	F	38	+ 6	190	97.2	68	5.7	170	8.1	Subnormal intelligence, various complaints such as chills and headaches. Diagnosis: Constitutional inadequate.
64204	F	47	- 9	134	98.0	76	8.0	210	6.7	Many symptoms, tired, numb all over etc. No physical abnormalities. Diagnosis: Psychoneurosis and hypochondriasis.
49470	F	60	- 0	123	98.0	68	8.1	250	6.5	Many fleeting pains, insomnia, palpitation. No physical abnormalities. Diagnosis: Anxiety state.
16204	M	24	+ 1	183	98.0	60	7.4	164	7.1	Jaundice 5 days. Liver enlarged and tender. Diagnosis: Infectious hepatitis.
A 582	F	54	+ 10	104	99.0	96	7.0	306	7.1	Loss of weight and appetite, jaundice, mass in abdomen. Diagnosis: Carcinoma of pancreas.
59200	F	64	+ 8	137	99.0	80	5.2.	-	7.2	Insomnia, palpitation, indigestion. No physical abnormality. Diagnosis: Psychoneurosis.
A 135	F	75	+ 56	109	98.8	64	4.0	-	5.6	Loss of weight and strength 2 years. Liver and spleen enlarged. W.B.C. 108,000. Diagnosis: Myelogenous leukemia.
A1094	F	56	+ 15	130	98.4	80	5.1	220	7.1	Congestive failure and diabetes for 1 year. Now improved. B.P. 175/125. Diagnosis: Diabetes Mellitus and essential hypertension.

CHAPTER IV

RESULTS

1. Comparison of various groups. The results of the total plasma magnesium estimations and the diffusible and non-diffusible fractions for the various groups are recorded in Tables XIV, XV and XVI. Because it is customary to express the non-diffusible or "bound" fraction as a percentage of the total this also has been calculated. The mean results together with their range and standard deviation for the various groups are reported in Table XVII. It is apparent that the "bound" fraction was consistently in the region of 50 per cent of the total and showed no significant difference in either level or in percentage relationship to the total in the various groups. The values for the total plasma magnesium also were similar for the various groups, the highest being found in patients with hypothyroidism.

2. Effect of treatment on percentage bound magnesium. The "bound" fraction of plasma magnesium was measured in two patients with hyperthyroidism and in two patients with hypothyroidism before and after they had received treatment. In each case the basal metabolic rate had returned to normal when the post-therapy estimation of magnesium was made. The results are recorded in Table XVIII and indicate that the percentage of "bound magnesium in plasma of patients with thyroid disease is not altered significantly with treatment.

TABLE XIV. PLASMA MAGNESIUM OF NORMAL SUBJECTS

(in mgm.%)

Number	Total	U.F.	Bound	% Bound
1	2.71	1.48	1.23	45.4
2	2.65	1.15	1.50	56.5
3	2.51	1.13	1.38	55.0
4	2.58	1.14	1.44	55.9
5	2.58	1.41	1.17	45.4
6	2.56	1.45	1.11	43.0
7	2.66	1.20	1.46	54.9
8	2.63	1.31	1.32	50.1
9	2.50	1.30	1.20	48.0
10	2.58	1.27	1.31	50.8
11	2.67	1.33	1.34	50.2
12	2.60	1.20	1.40	53.9

TABLE XV. PLASMA MAGNESIUM OF SUBJECTS WITH THYROID
DISEASES.
(in mgm.%)

Group	Case Number	Total	Ultra-filtrate	Bound	% Bound
HYPERTHYROID	A4051	2.70	1.55	1.15	42.6
	16051	2.41	1.40	1.01	41.8
	16383	2.50	1.25	1.25	50.0
	15562	2.23	1.15	1.08	48.5
	16469	2.73	1.24	1.49	53.6
	6900	2.75	1.40	1.35	49.1
	A5491	2.66	1.14	1.52	57.1
	A6017	2.80	1.46	1.34	47.9
	16469	2.43	1.35	1.08	44.5
NON-TOXIC NODULAR THYROID	15420	2.66	1.26	1.40	52.6
	64344	2.68	1.25	1.43	53.3
	15814	2.35	1.08	1.27	54.0
	15865	2.55	1.13	1.42	55.7
	5539	2.48	1.33	1.15	46.4
	A981	2.70	1.44	1.26	46.7
HYPOTHYROID	15166	2.96	1.40	1.56	52.6
	13555	2.56	1.20	1.36	53.1
	16004	2.60	1.20	1.40	54.0
	16306	2.58	1.20	1.38	53.5
	16205	2.66	1.33	1.33	50.0
	A1075	2.58	1.20	1.38	53.5
	A486	3.03	1.54	1.49	49.2
	A345	3.05	1.32	1.73	56.6
	13592	2.78	1.35	1.43	51.4
	10618	2.79	1.47	1.32	47.4

TABLE XVI. PLASMA MAGNESIUM OF MISCELLANEOUS SUBJECTS

(in mgm.%)

Case Number	Diagnosis	Total	U.F.	Bound	% Bound
10818	Congestive Heart Failure	2.51	1.50	1.01	40.2
15714	Hypertensive Heart Failure	2.73	1.46	1.27	46.5
15385	Paget's Disease	2.33	1.13	1.20	51.5
Child	Behaviour Problem	2.50	1.34	1.16	46.5
9604	Psychotic	2.98	1.33	1.65	55.3
47631	Hypertension	2.73	1.25	1.48	54.2
63593	Psychoneurosis	2.55	1.20	1.35	53.0
64204	Psychoneurosis	2.51	1.15	1.36	54.1
49470	Psychoneurosis	2.48	1.27	1.21	48.8
16204	Infectious Hepatites	2.83	1.30	1.53	54.0
A 582	Ca. Pancreas	2.83	1.07	1.76	62.1
59200	Headache	2.95	1.15	1.80	61.0
A 135	Myelogenous Leukemia	2.65	1.30	1.35	51.0
A1094	Diabetes C.H.F.	2.68	1.20	1.48	55.2

TABLE XVII. PLASMA MAGNESIUM IN VARIOUS GROUPS

(in mgm. %)

Group	Number of Cases	Mean Total ± S.D. and range	Mean Diffusible ± S.D. and range	Mean "Bound" ± S.D. and * range	Mean % "Bound" ± S.D. and ** range
Normal	12	2.60 ± .06 (2.50 - 2.71)	1.28 ± .12 (1.13 - 1.48)	1.30 ± .12 (1.11 - 1.50)	50.2 ± 4.6 (43.0 - 56.5)
Miscellaneous	14	2.66 ± .19 (2.33 - 2.98)	1.26 ± .12 (1.07 - 1.50)	1.13 ± .22 (1.01 - 1.80)	52.3 ± 5.7 (40.2 - 62.1)
Hyperthyroid	9	2.58 ± .19 (2.23 - 2.80)	1.32 ± .12 (1.14 - 1.55)	1.25 ± .19 (1.01 - 1.52)	48.3 ± 4.9 (41.8 - 57.1)
Non Toxic Nodular Thyroid	6	2.57 ± .14 (2.35 - 2.70)	1.24 ± .13 (1.08 - 1.44)	1.32 ± .11 (1.15 - 1.43)	51.4 ± 3.9 (46.4 - 55.7)
Hypothyroid	10	2.75 ± .19 (2.56 - 3.05)	1.32 ± .11 (1.20 - 1.54)	1.43 ± .12 (1.32 - 1.73)	52.1 ± 2.7 (47.4 - 56.6)

* Difference between total and diffusible

** Percentage of total

TABLE XVIII. EFFECT OF TREATMENT ON PERCENTAGE OF BOUND MAGNESIUM
IN PLASMA OF PATIENTS WITH THYROID DISEASE.

Condition	Subject	Before treatment	After treatment
Hyperthyroidism	A5491	57.1	54.0
	A6017	47.9	45.7
Hypothyroidism	10618	47.4	46.5
	13592	51.4	48.0

CHAPTER V

DISCUSSION

1. Total plasma magnesium values. In considering the above results it should be noted that the values for the total plasma magnesium are slightly higher than those found by Kunkel et al. (1947) who also used the titan yellow method. This may be due to the difference in standard solutions. However, the values reported here are well within the accepted range of 2 to 3 milligrams per cent. It is of interest that the values for total magnesium in the hypothyroid group were slightly higher than in the normal group. This may have some relation to hypothermic states. Suomalainen (1938) reported evidence which showed that total serum magnesium was raised in hibernating animals. Similarly, Steadman et al. (1943) showed that total serum magnesium was raised in rabbits whose body temperatures were lowered by experimental means. Recently Sunderman and Haymaker (1947) described a case with a lesion of the hypothalamus which was associated with a high total serum magnesium and subnormal temperature. Since patients with myxedema frequently have subnormal body temperatures it is possible that there exists some relationship between the high total plasma magnesium in this condition and the hypothermic state. However, this implication can not be confirmed by the above results.

2. "Bound" magnesium values. The "bound" fraction of plasma magnesium was found to be approximately 50 per cent of the total plasma magnesium. This is significantly higher than the values reported by Soffer et al. (1939, 1941), Dine and Lavietes (1942), and Bissell (1945), and at the upper level of the range found by Cope and Wolff (1942) (See Table VI).

3. Reasons for difference of results. The ultrafiltration apparatus used in this investigation has given very consistent results. This is in contrast with the results of the above investigators who reported values which had a wide range in both normal and hyperthyroid groups. The use of cellophane as a membrane for ultrafiltration has resulted in consistent results for diffusible calcium which is in the region of 60 per cent (Schmidt and Greenberg 1935). In addition, both previous investigation and the work presented in this study has established that a variation of many factors does not alter the concentration of diffusible ions in the ultrafiltrate. Therefore, the variation in results concerning diffusible magnesium must indicate a fundamental difference in techniques or methods of estimating plasma magnesium. The above authors used the method of Briggs (1922) or some modification of it to determine the magnesium concentration. As has been discussed on page 6, this method necessitates the precipitation of calcium before the subsequent estimation of magnesium. This precipitation may affect the

subsequent determination of magnesium. Also, the blue color formed by this method is very unstable and many workers in other fields have found it difficult to obtain consistent results. In the titan yellow method as used in this investigation, calcium in the concentration found in human plasma does not affect the estimation of magnesium (Garner 1946). This may be the explanation for the variance of results.

4. Relationship of thyroid function and the metabolism of magnesium. It is apparent from this study that disorders of the thyroid gland do not alter the concentration of the non-diffusible fraction of plasma magnesium as was claimed by Soffer et al. (1939, 1941) and Dine and Lavietes (1942). Soffer et al. supported their claim with results from experiments on thyroidectomized dogs who had low concentrations of non-diffusible plasma magnesium. They showed in these dogs that the non-diffusible or "bound" fraction could be increased by substitution therapy with thyroxine. But in two hyperthyroid and two hypothyroid patients treated in this study until their basal metabolic rates were normal no such change was demonstrated. Therefore, at least in humans, the bound magnesium of plasma does not appear to be affected by therapy. In the introduction to this study mention was made of some direct evidence and of additional indirect evidence which indicated the possibility of an association between the metabolism of magnesium and function of

the thyroid gland. However, the results of the present investigation have shown that in disorders of thyroid function neither the concentration of plasma magnesium nor the distribution of its fractions was altered. Nevertheless, it is possible that the metabolism of magnesium in other tissues may be governed by the function of the thyroid gland. Therefore, it can only be concluded that as far as the concentration of plasma magnesium is a reflection of magnesium metabolism there appears to be no relationship with thyroid function.

SUMMARY AND CONCLUSIONS

1. A review of the literature indicates that magnesium is important in many metabolic processes of the animal organism and that further investigation will be necessary to elucidate the mechanism of these processes.
2. The recently reported titan yellow method for estimating magnesium in biological fluids has been applied and found to be accurate when measuring the magnesium concentration of human plasma.
3. Magnesium determinations were made on the plasma and the ultrafiltrate from such plasma of 12 normal individuals, 14 patients with miscellaneous diseases, 10 with myxedema, 6 with non-toxic goitre and 9 with hyperthyroidism. The non-diffusible fraction of plasma magnesium was found to be approximately 50 per cent of the total and was not altered by thyroid dysfunction.
4. It is concluded that disorders of the thyroid gland do not alter the concentration of the total plasma magnesium or the distribution of its fractions.

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