its capacity to activate prothrombin. In further characterization, other properties have been described for thrombokinase prepared from plasma(7). It was not sedimented appreciably in 2 hours at 85,000 g, and thus differed from certain easily sedimentable thromboplastins. It converted prothrombin to thrombin in presence of excess oxalate, but was much more effective when accompanied by platelets (or cephalin) plus calcium. Activation of prothrombin by thrombokinase was suppressed by soy bean trypsin inhibitor.

It is now added that purified thrombokinase has shown esterase activity of the order of 337 TAMe units/mg protein. This is more than half that reported for highly purified thrombin and several times that reported for highly purified plasmin(4). No conclusions are drawn regarding relative purity of these preparations, since degree of TAMe esterase activity characteristically varies from one enzyme to another.

These properties of thrombokinase may facilitate comparison with other preparations of clotting factors, purified by electrophoresis (8,9).

Summary. Thrombokinase, purified from 738 liters of bovine plasma, was subjected to repeated fractionations by continuous flow paper electrophoresis. This increased the specific activity of kinase and led to a 3rd electrophoretic run which placed almost all the protein in a single peak. Kinase activity was closely accompanied by TAMe esterase activity through successive stages of electrophoretic fractionation. The kinase fractions of the 3rd run showed esterase activity of the order of 337 TAMe units/mg protein. The results are in accord with the view that TAMe is a substrate for thrombokinase and that thrombokinase is a trypsin-like enzyme.

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Received November 23, 1959. P.S.E.B.M., 1960, v103.

Effect of Glucose and Insulin on Magnesium Metabolism in Rabbits. A Study with Mg^{28}.*† (25520)

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The factors regulating metabolism of magnesium are not well understood, although previous studies have suggested that it may be related to the metabolism of carbohydrate and insulin(1,2). Tissue distribution of radioactive magnesium (Mg^{28}) following its intravenous administration into normal rabbits has been described(3). Our purpose was to determine whether administration of glucose and insulin would alter the exchange of Mg^{28} in rabbit tissues.

Material and methods. Normal domestic adult rabbits of both sexes, with initial body weights between 2 and 4 kg were kept in individual stainless steel metabolism cages and fed a stock diet of compressed pellets. Water was given without restriction. Commercial zinc *insulin* crystals (USP), 40 units/ml, and a sterile 5% solution of *dextrose* in distilled water were used. Mg^{28} was received as $MgCl_2$

* Supported by contract between Univ. of Colorado and U. S. Atomic Energy Comm. and grant-in-aid from Am. Heart Assn. and Colorado Heart Assn.
† Mg^{28} was supplied by Brookhaven National Lab.

in concentrated HCl. 200 μc were contained in 25 to 30 meq of stable magnesium. The material was neutralized with 1 N NaOH and then diluted in physiologic saline solution to contain 0.2 meq of Mg/ml. *Serum* and *urine magnesium* determinations were performed by modification of the molybdivanadate method for phosphate(4). Tissue magnesium determinations were performed by the method of Stutzman(5). Radioactivity assay. Samples of plasma and tissues were assayed for gamma ray activity with a scintillation counter. A total of 10,000 counts were made on each sample. All determinations were corrected for physical decay of the isotope. *Plan of experiments.* A tracer dose of Mg^{28} contained in 1 to 2 meq of stable magnesium was injected intravenously into each animal, and followed by a dose of insulin, glucose, or both. Blood was collected by cardiac puncture or from marginal ear vein into heparinized test tubes. After animal was killed by air embolism, radioactivity content of various tissues was compared with that of plasma obtained at time of death. Chemical determinations for stable magnesium were performed on same samples of tissues that had been assayed for radioactivity. Relative activity of tissue was calculated as follows:

$$\text{Relative activity} = \frac{\text{cpm/g (wet wt) of tissue}}{\text{cpm/ml of plasma}}.$$

In a preliminary study, 6 rabbits which had received the tracer dose of tagged magnesium were each given, by injection into interscapular region, 5 units of insulin/kg of body weight. All animals began to have convulsions within 1 to 1½ hr. Infusion of 5 to 10 ml of a 5% solution of dextrose in water stopped the convulsions. Injections of dextrose were subsequently given at intervals of 30 to 60 minutes as animals appeared overexcited. Animals were killed in pairs at intervals of 2, 4 and 6 hr after injection of insulin and the following tissues were analyzed for radioactivity content: skin, heart, liver, muscle, bone, appendix, kidney and adrenal. When results of these studies were compared with those obtained in normal rabbits which had received tagged magnesium only(3), all animals given insulin and dextrose showed an increase in relative activity of all tissues obtained; relative activities were highest, however, at 4 hr. For this reason, tissues studied in the definitive experiment were obtained 4 hr after initial injection. In this experiment, observations were made on 32 rabbits divided into 4 groups of 8. Animals in *Group A* received only tracer dose of Mg^{28} in 1 to 2 meq of *magnesium*. In *Group B,* all 8 rabbits were given, in addition to tagged *magnesium, insulin* and *glucose*. Four animals in this group were given a single interscapular injection of insulin, 5 units/kg of body weight, followed by 3 doses of dextrose, each 5 ml of 5% solution in water, administered interscapularly at hourly intervals. In the other 4 rabbits, 2 units of insulin/kg were given intravenously, followed immediately by one intravenous injection of 10 ml of 5% dextrose in water. Four rabbits in *Group C* were given, in addition to tagged *magnesium,* an interscapular injection of *insulin,* 5 units/kg body weight, and 4 others were given intravenous injection of *insulin,* 2 units/kg. Besides the tagged *magnesium,* each animal in *Group D* received *dextrose* parenterally. Four were given 10 ml of a 5% solution of dextrose in water intravenously a few minutes after injection of magnesium. Four others were given 3 interscapular injections, each containing 5 ml of 5% dextrose in water, at intervals of one hr.

Results. Group A. Relative radioactivity of the various tissues studied is given in Table I, and magnesium content of tissues is summarized in Table II. *Group B.* Administration of insulin and dextrose resulted in a significant increase in relative radioactivity of all tissues studied. No significant change, however, was found in concentrations of blood glucose or serum magnesium. Significant increases occurred in concentrations of stable magnesium in the heart and the skeletal muscle. *Group C.* Injection of glucose resulted in significant increases in blood glucose concentration and relative radioactivity of the heart. Magnesium content of heart and muscle was significantly elevated. *Group D.* Following administration of insulin alone, the sole change was a significant increase in magnesium content of the heart.

TABLE I. Effect of Insulin and Glucose on Relative Mg^{28} Activity in the Rabbit.

Tissue		Mg (control)	Animals inj. with Mg, insulin & dextrose	Mg & dextrose	Mg & insulin
Bone	$\bar{x}$	12.90	22.10*	18.10	40.50
	$s_{\bar{x}}$	1.13	2.17	1.65	18.10
Kidney	$\bar{x}$	11.00	14.60*	13.20	22.20
	$s_{\bar{x}}$	.60	.94	1.31	6.95
Heart	$\bar{x}$	5.90	12.60*	11.20*	19.40
	$s_{\bar{x}}$	.99	.37	.72	6.05
Liver	$\bar{x}$	5.10	9.80*	7.40	15.40
	$s_{\bar{x}}$	.42	.44	1.25	4.82
Appendix	$\bar{x}$	3.90†	9.90*	7.93	12.78
	$s_{\bar{x}}$	.23	.87	.85	2.57
Skin	$\bar{x}$	1.25	2.34*	2.06	3.56
	$s_{\bar{x}}$	.14	.15	.42	.87
Muscle	$\bar{x}$	.50	1.24*	.89	2.30
	$s_{\bar{x}}$	.10	.14	.09	.82
Serum Mg‡	$\bar{x}$	1.81	1.70	2.03	2.09
	$s_{\bar{x}}$	.33	.09	.09	.08
Blood glucose‖	$\bar{x}$	82.3	69.9	97.1 *	§
	$s_{\bar{x}}$	3.93	4.85	2.28	

$\bar{x}$ = mean value expressed as relative activity, based on data from 8 rabbits.
$s_{\bar{x}}$ = stand. error of mean.
* Significant difference when compared with mean control value. P = less than 0.01.
† Mean of 4 animals.
‡ meq/l.
§ Not done.
‖ mg/100 ml.

Comments. Rate of uptake of Mg^{28} by bone appears to vary with the species studied; Mg^{28} is concentrated slowly in bones of human beings and dogs(6), whereas in the rabbit Mg^{28} is absorbed more rapidly by bone than by any other tissue studied. The next highest relative activities were found in kidneys, heart and liver, while skeletal muscle showed slowest uptake. Magnesium content of bone (300 meq/kg) was at least 13 times higher than that of muscle (22 meq/kg), the tissue with the next highest magnesium content.

Previous studies on rabbits have shown that parenteral administration of magnesium may cause a temporary hyperglycemia or hypoglycemia, depending upon dosage given(2). Intravenous injection of small amounts of magnesium decreases rate of removal of glucose from the blood stream, probably because of impaired peripheral utilization, accelerated glycolysis, or both. Intravenous administration of insulin may result in temporary increase in serum magnesium concentration, followed by a decrease(1). Glucose infusions have been reported to decrease serum magnesium concentrations(5). Insulin and magnesium are said to have a synergistic effect (7).

In the present study, although low specific activity of the isotope made it necessary to subject the rabbits to a slight magnesium load, no significant change in serum magnesium was found when values at 4 hours were compared with serum magnesium concentrations previously obtained in uninjected rabbits(3). While simultaneous injection of magnesium, insulin and glucose did not alter significantly serum magnesium concentration or blood glucose level, it did increase uptake of Mg^{28} in all tissues studied. Since magnesium content of bone, liver and skin did not increase significantly, the increase in Mg^{28} uptake by these tissues is probably due to more rapid exchange of Mg^{28} between their intracellular and extracellular compartments. In skeletal muscle and heart, on the other hand, there was a significant increase in magnesium content as well as in the Mg^{28} relative activity. In these 2 locations, therefore, it is concluded

TABLE II. Effect of Insulin and Glucose on Magnesium Content of Rabbit Tissues.

Tissue		Mg (control)	Animals inj. with Mg, insulin & dextrose	Mg & dextrose	Mg & insulin
Bone	$\bar{x}$	299.5	292.6	309.7	334.4
	$s_{\bar{x}}$	8.92	21.30	6.07	17.70
Muscle	$\bar{x}$	21.5	28.6*	29.8*	20.5
	$s_{\bar{x}}$	.49	1.46	1.17	1.57
Heart	$\bar{x}$	13.0	23.5*	25.9*	16.3*
	$s_{\bar{x}}$	.40	2.21	1.93	.39
Liver	$\bar{x}$	12.4	14.2	13.4	14.6
	$s_{\bar{x}}$	.50	.40	.35	.67
Kidney	$\bar{x}$	12.2	†	12.3	12.0
	$s_{\bar{x}}$	.65		.76	.73
Skin	$\bar{x}$	6.4	5.6	5.2	5.1
	$s_{\bar{x}}$	.26	.31	.37	.48

$\bar{x}$ = mean value expressed as meq of Mg/kg (wet wt) of tissue, based on data from 8 rabbits.
$s_{\bar{x}}$ = stand. error of mean.
* Significant difference when compared with mean control value. P = less than 0.01.
† Not determined.

that actual deposition of increased amounts of magnesium had occurred.

Less striking changes were observed when either insulin or dextrose alone was given following injection of magnesium. It is of interest that magnesium content of both heart and skeletal muscles was increased by administration of dextrose and magnesium, while the sole significant increase in magnesium content following injection of insulin and magnesium occurred in the heart. The combination of insulin and magnesium resulted in the highest mean relative Mg^{28} activities observed in the 4 groups, but the range of values was so wide that no statistically significant differences were elicited.

The biochemical mechanisms whereby dextrose and insulin accelerate Mg^{28} turnover in the tissue are obscure. Animals in this study were subjected to a slight magnesium load. Previous studies indicated that magnesium increases glycogenesis in liver slices(8) and rat-heart slices(9), activates phosphatases from kidneys, intestinal mucosa, liver, bone and plasma, and increases activity of ATP(10). Although it is not known at the present time whether these enzymatic processes are involved in turnover of magnesium, it is of interest that these are the very tissues which in this study showed increased Mg^{28} activity in the presence of insulin and glucose.

Taken as a whole, the observations made in this study and previous studies suggest that metabolism of magnesium is intimately related to that of carbohydrate and that the turnover of magnesium in tissues is regulated by insulin and dextrose. The relationship between metabolism of magnesium and that of other intracellular electrolytes, such as potassium, remains to be studied.

Summary. In rabbits which had received by intravenous injection 1 to 2 meq of magnesium tagged with Mg^{28}, parenteral administration of insulin and dextrose significantly accelerated Mg^{28} uptake in bone, kidney, heart, liver, appendix, skin and skeletal muscle. Tissue magnesium content was increased in heart and skeletal muscle. Less striking changes were noted when insulin or dextrose alone was given following injection of magnesium. Magnesium metabolism appears intimately related to that of carbohydrate and insulin.

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Received November 25, 1959. P.S.E.B.M., 1960, v103.

Effect of ACTH on Regeneration of Adrenal Cortex Following Autografting in Hyophysectomized Rats.*† (25521)

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Although adrenal cortical tissue may survive autogenous transplantation in the hypophysectomized rat, regeneration is less than in rats having intact pituitary glands. ACTH treatment of hypophysectomized rats did stimulate regeneration of cortical tissue in

* This research was supported by USPHS grant.

† Grateful acknowledgement is made of assistance of Dr. George F. Wilgram in fixing and microscopic examination of tissues.