females were given a supplement of tocopherol throughout the gestation period the blood cells of the young rats were protected. The possible application of this hemolysis method to the bioassay of vitamin E has been discussed.

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Experimental Control of Serum Calcium Levels *in Vivo*.* (17924)

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The constancy of the total calcium level of the blood is the result of the interplay of numerous factors which include calcium intake, absorption and excretion, Vitamin D economy, parathyroid gland function, and blood phosphate, bicarbonate, protein and pH levels(1). The relations which determine the distribution of the total blood calcium between the ionic and the bound forms are largely unknown. It is generally accepted that the ionic calcium in the blood is the physiologically active form. Were it possible to control the ionic blood-calcium levels by exogenous means further insight into the relationships and mechanisms which govern calcium levels and distribution might be obtained. Soluble oxalates lower blood calcium ions by precipitation of insoluble calcium oxalate. Citrates, tartrates, phthalates, fluorides, polyphosphates, glycine, and other anions lower blood calcium ion by the formation of soluble nonionic complexes(2). The characteristic physiological sequelae attributable to hypocalcemia follow the administration of these agents. However, the use of these materials for quantitative control of calcium ion *in vivo* is subject to certain experimental difficulties. Ethylenediamine tetra-acetic acid (E.D.T.A.A.) forms soluble nonionized complexes with many bivalent cations(3) and behaves as a chelating agent. Dyckerhoff *et al.*(4) have reported that E.D.T.A.A. inhibits blood coagulation *in vitro* probably as the result of the removal of calcium ion by complex formation. At physiological pH we have found that in human or rabbit serum the complex of calcium ion with E.D.T.A.A. forms stoichiometrically. Calcium ion bound in E.D.T.A.A. complex is not precipitated by the addition of oxalates and hence the total remaining non-complexed calcium which may consist of both ionic and protein bound calcium can be determined by oxalate precipitation. We have demonstrated this by numerous *in vitro* experiments. That the same combination occurs *in vivo* is illustrated in Fig. 1 where the physiological action of E.D.T.A.A. was shown to be the consequence of its combination with calcium ion. Rapid (20 sec.) intravenous injection into the marginal ear vein of rabbits of E.D.T.A.A. as the neutral sodium salt, 100 mg/kg, resulted in immediate lowering of the available calcium level with consequent death by hypocalcemic tetany. When the calcium chelation of E.D.T.A.A. has been saturated by admixture with stoichiometric quantities of calcium in the form of its chloride or gluconogluconate salt prior to injection, the previously demonstrated toxic effects did not occur (Fig. 1). A second injection of the neutral sodium salt of E.D.T.A.A. 100 mg/kg, then caused

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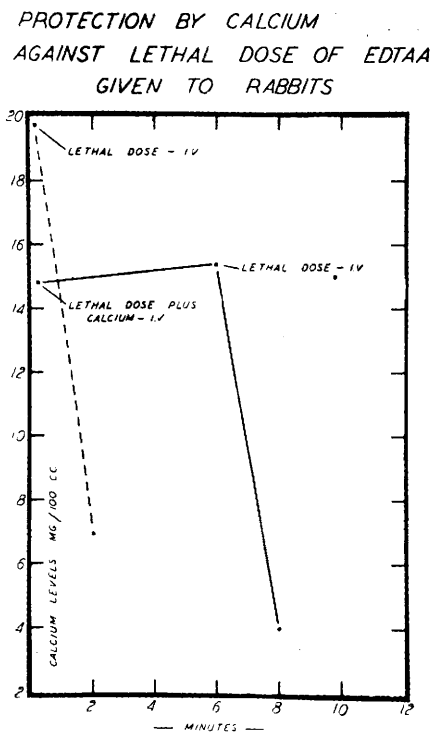

FIG. 1.

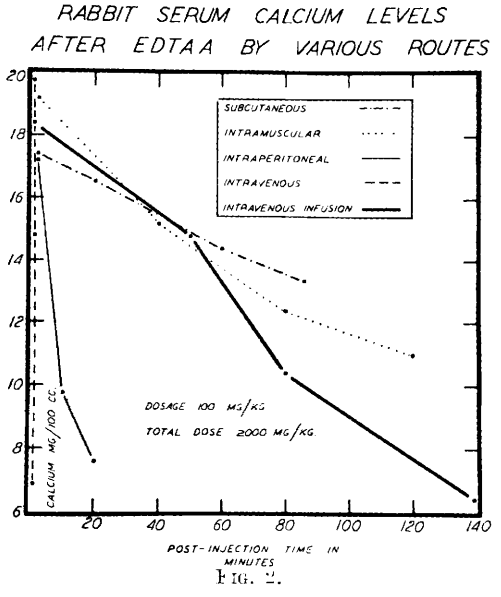

FIG. 2.

death of the rabbit in the typical manner. In numerous experiments it has been demonstrated that complete recovery from the effects of E.D.T.A.A. could be brought about by intravenous or intracardiac administration of calcium salts at any time prior to death. The rapidity with which the calcium level is lowered is a function of the mode of administration of the chelating agent (Fig. 2) and the rate at which it is injected as well as the dose. Intravenous, intraperitoneal, intramuscular and subcutaneous injection, as might be anticipated, resulted in successively diminishing rates of lowering of the available calcium serum levels. By slow intravenous drip it was possible to inject 2,000 mg per kilo of E.D.T.A.A. over a period of 3 hours before the serum calcium fell to a fatal level. By controlling the rates and amounts of chelate given it was possible to lower the available calcium serum level and maintain this level by balancing the complex formation against replenishment of blood calcium from the mobile reserves of the animal. That E.D.T.A.A. may be absorbed through the skin is illustrated in Fig. 3. Daily applications of a 5% water-soluble ointment to 2 human subjects resulted in a gradual fall of the serum calcium to 8 mg per 100 cc in a period of 4 days. Intravenous administration of calcium gluconogluconate resulted in the re-establishment of normal calcium levels.

Magnesium ion may have a regulatory action on calcium metabolism as has been suggested by Mendel and Benedict(5) and Becka(6). More direct evidence for this

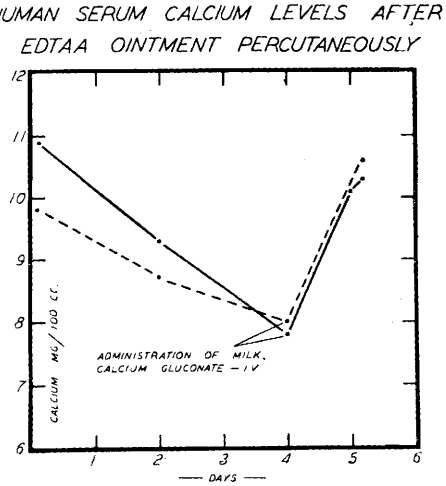

FIG. 3.

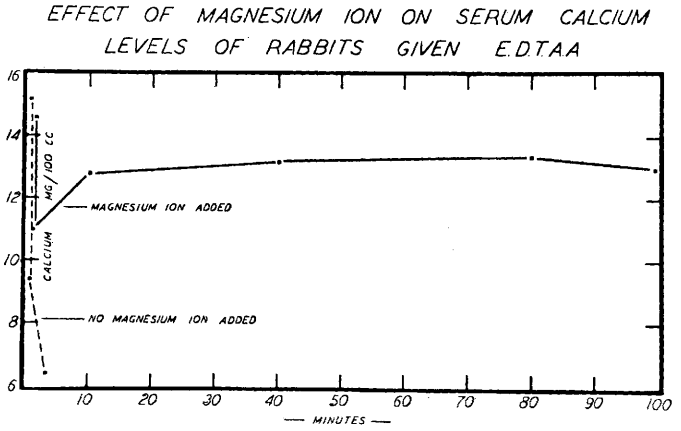

FIG. 4.

function than hitherto available is presented in Fig. 4. It can be noted that simultaneous administration of magnesium ion with an ordinarily lethal dose of E.D.T.A.A. resulted in a rapid replenishment of the blood calcium levels. This effect was noted whether the magnesium was pre-added to the E.D.T.A.A. solution with formation of the magnesium complex of E.D.T.A.A. prior to injection or if magnesium ion in the form of the sulfate was given simultaneously into the opposite ear vein of the rabbit or was injected directly into the heart simultaneously with the intravenous injection of E.D.T.A.A. in the ear vein. That the result was not due to combination of E.D.T.A.A. with magnesium ion rather than calcium ion has been indicated by the demonstration that the complex of calcium ion by E.D.T.A.A. as measured by the precipitation of unbound calcium by oxalate did not change in the presence of magnesium ion in the system when tested *in vitro*. Confirmation of this work was reported in a study by physical means of the preferential complex formation of E.D.T.A.A. with various bivalent cations(7). In the physiological pH range E.D.T.A.A. combines with calcium ion preferentially over magnesium ion.

Summary. The ability of ethylenediamine tetra-acetic acid to form undissociated calcium complexes at physiological pH has been utilized as a tool to regulate available serum calcium levels *in vivo*. Further evidence has been presented for the regulatory action of magnesium ion on serum calcium levels.

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Effect of Physical Factors on Radiosodium Clearance from Subcutaneous and Intramuscular Sites in Animals.* (17925)

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This experimental study was based upon the recent work of Kety(1,2,3) and Elkin *et*

* Presented before the American Physiological Society, April 21, 1950.

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