intake during the weeks in which the propylene glycol-containing diets were fed. At the highest level of intake, more than 25% of the ingested propylene glycol was accounted for in the urine while in no instance was more than 1.5% of the ingested glycerol accounted for. This finding may partially explain an earlier observation that glycerol-fed rats made the greatest body weight gains per caloric unit of food intake.

While those groups of rats receiving the glycerol-containing diets gave birth to a greater number of litters and a greater number of young per litter, they were also more successful in weaning a higher percentage of the young than were similar groups of rats subsisting on comparable propylene glycol-containing diets. Reproduction and subsequent rearing of young were abnormally low with all groups of animals, including those on the purified basal ration. An attempt was made to correct this situation by addition of vitamin and mineral supplements without success. Consequently, further attempts to continue reproduction studies with purified diets were abandoned.

Reproduction studies with diets composed of the breeding colony diet (70%) and glycerol (30%) or propylene glycol (30%) resulted in some very significant differences. Rats which have received the glycerol-containing diet are now in the fifth generation, while second generation young have not yet been successfully weaned by the propylene glycol fed mothers. A more extensive series of diets which contain proteins, minerals, and vitamins from natural food sources and in which part of the energy is furnished as glycerol or as propylene glycol is now under investigation. A report on this phase of the investigation, together with the details of the studies herein reported, including pathological findings, will be forthcoming.

The concentrations of glycerol and propylene glycol in some of these diets were much greater than concentrations ordinarily ingested, but the information obtained may have a pertinent bearing on the relative toxicity of the two polyhydric alcohols.

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Comparative Effectiveness of Calcium and Strontium in Plasma Coagulation.

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The classic work of Arthus and Pagés[1] and Ringer and Sainsbury[2] established the importance of calcium for blood coagulation. These investigators also tried to replace this ion by strontium. They found that this element is less effective in producing blood clotting than calcium. Their experiments were of a qualitative nature, and the subject was later studied under more quantitative conditions by Mallenby,[3] using bird plasma, and by Heard,[4] using oxalated cat blood. Both found that the coagulation time with strontium was considerably longer than with equivalent amounts of calcium. Woehler[5] has reviewed further work on this subject. It appears, however, that no comparative study has been undertaken on human blood plasma with the more quantitative methods developed in recent years.

Methods. The following experiments were conducted on the oxalated plasma of seven

[1] Arthus, M., and Pagés, C., *Arch. de Physiol.*, 1890, **12**, 739.

[2] Ringer, S., and Sainsbury, H., *J. Physiol.*, 1890, **11**, 369.

[3] Mallenby, J., *J. Physiol.*, 1909, **38**, 28.

[4] Heard, W. N., *J. Physiol.*, 1917, **51**, 294.

[5] Woehler, E., *Erg. d. Physiol.*, 1929, **28**, 443.

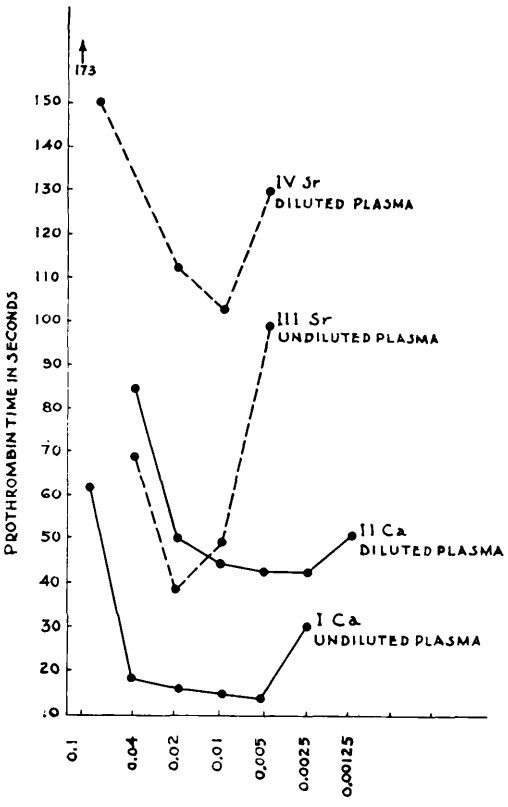

FIG. 1.

healthy humans using the method of Quick[6] for the determination of the prothrombin times. Both undiluted plasma and diluted plasma in a concentration of 12.5% were used, since it is known that diluted plasma tends to magnify minor changes in prothrombin time which are not as evident in the undiluted plasma. For the determination 0.1 cc of plasma was mixed with 0.1 cc of a suspension of thromboplastin prepared from rabbit brain by acetone desiccation. To this mixture, maintained at 37.5°C in a water bath, 0.1 cc of calcium chloride or strontium chloride in various molar concentrations was added. The mixture was continually stirred with a nichrome wire until clotting occurred.

Results and Discussion. The data summarized in Fig. 1 show the curves constructed from the averages of the determinations. The effect of varying the calcium concentration upon the prothrombin time of concentrated chicken plasma has been studied by Quick.[7] He found the shortest prothrombin time with the addition of a 0.005 and 0.0025 molar $CaCl_2$. Pohle and Stewart[8] conducted similar studies with undiluted human plasma. They found a prothrombin time of 11 seconds with 0.01 molar $CaCl_2$. The shortest prothrombin time was 10 seconds with 0.005 molar $CaCl_2$, while with 0.0025 molar $CaCl_2$ the prothrombin time was again slightly longer, 10.5 seconds. The prothrombin time became markedly prolonged with higher or lower concentrations. Prothrombin times in our experiments were slightly longer at the corresponding calcium levels, which was probably due to the use of a somewhat less potent thromboplastin. Our shortest prothrombin time occurred with 0.005 molar $CaCl_2$ concentration, the same as given by Pohle and Stewart.[8] However, the slope of our curve in the lower concentration range is steeper. For instance, the prothrombin time is already 30 seconds with 0.0025 molar $CaCl_2$. Apparently the effect of different $CaCl_2$ concentrations upon the prothrombin time of diluted plasma has not been reported. The curve constructed from the values obtained with 12.5% plasma is similar in shape to the one of the concentrated plasma, but the concentration range in which the clotting time shows but slight change is shifted to the lower calcium concentrations.

If we compare these findings with the data obtained with $SrCl_2$, we notice that the prothrombin times are considerably longer than those with $CaCl_2$, and that the optimal $SrCl_2$ concentration is much higher for both the undiluted and the diluted plasma. These optimal concentrations of $SrCl_2$ are 0.02 molar for whole plasma and 0.01 molar for diluted plasma. The range of concentrations in which the prothrombin times show but slight change is much smaller for strontium than for calcium, the curve rising sharply on either side

[6] Quick, A. J., *The Hemorrhagic Diseases and the Physiology of Hemostasis*, Charles C. Thomas, 1942.

[7] Quick, A. J., *Am. J. Physiol.*, 1940, **131**, 455.

[8] Pohle, F. J., and Stewart, J. K., *Am. J. Med. Sci.*, 1939, **198**, 622.

of this zone. This is interpreted as a further indication of the greater specificity of calcium as compared with strontium for plasma coagulation.

We have conducted a number of additional studies using 12.5% rat plasma and the method of prothrombin determination as modified by Link and collaborators.[9] Due to the fact that individual rats vary from each other in their normal prothrombin time more than the humans do, the results are not as conclusive. However, our findings indicate that here also the prothrombin times with $SrCl_2$ were considerably longer than with $CaCl_2$. As in human plasma, no marked changes in prothrombin time occurred with $CaCl_2$ between 0.02 and 0.005 molar, while with $SrCl_2$ the optimal concentration was at 0.01 molar with a rather steep rise of the prothrombin times with either 0.02 or 0.005 molar $SrCl_2$. It was also observed with the plasma of both humans and rats that the clot formed with $SrCl_2$ was not as firm as with $CaCl_2$, and this was the more evident the farther away the $SrCl_2$ concentration was removed from the optimum.

Summary. The comparative effectiveness of $CaCl_2$ and $SrCl_2$ as clotting agents in various concentrations was studied with undiluted and diluted human plasma. It was found that the prothrombin time with $SrCl_2$ is longer than with $CaCl_2$, and also that the optimal molar concentration of strontium is much higher. Further, the range of concentrations with which a relatively short prothrombin time can be obtained is much smaller with $SrCl_2$ than with $CaCl_2$. This is taken as further evidence for the greater specificity of the calcium ion. Corresponding experiments with diluted rat plasma gave essentially similar results.

[9] Campbell, H. A., Smith, W. K., Roberts, W. L., and Link, K. P., *J. Biol. Chem.*, 1941, **138**, 1.

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Efficacy of M-Amino Ethyl Benzoate as an Anesthetic for Amphibian Embryos.

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Chloretone, cocaine, novocaine, and freezing have been used as anesthetics for living embryos but have not proven to be entirely satisfactory. Rothlin[1] described a meta-aminobenzoic acid-ethylester in the form of methansulphonate (isomeric with anesthesin) which is highly soluble in water, 3 times less toxic than novocaine and 10 times less toxic than cocaine. This anesthetic had been used by Sandoz and Stryzowski,[2] Witschi,[3] Christensen,[4] Rotmann,[5] Glucksohn,[6] Goodrich and Nichols,[7], Baudin,[8] and Sato,[9] but these investigators were interested in it only as a tool.

This anesthetic is commercially known as M.S.222.* It has no effect on ciliary action or on the process of differentiation. Both cilia and muscle will develop in an embryo while under the influence of this anesthetic but the cilia alone will function. This not

[1] Rothlen, E., *Schweiz Med. Wochr.*, 1932, **45**, 1042.

[2] Sandoz, M., and Stryzowski, R., *Bull. Soc., Vand. Sc. Nat.*, 1920, **53**, 199.

[3] Witschi, E., *J. Exp. Zool.*, 1927, **47**, 269.

[4] Christensen, K., *Anat. Rec.*, 1931, **48**, 241.

[5] Rotmann, E., *Roux Arch. f. Entwicklungsmechanik*, 1931, **124**, 747.

[6] Glucksohn, S., *Roux Arch. f. Entwicklungsmechanik*, 1931, **125**, 341.

[7] Goodrich, H. B., and Nichols, R., *Anat. Rec.*, 1931, **51**, 513.

[8] Baudin, L., *C. R. Soc. de biol.*, 1932, **110**, 235.

[9] Sato, T., *Roux Arch. f. Entwicklungsmechanik*, 1932, **122**, 451.

* This anesthetic is available through M. Sandoz & Co., New York City.