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.

14725 P

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

BEULAH H. MCGOVERN AND ROBERTS RUGH.

From Washington Square College of Arts and Sciences, New York University.

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.

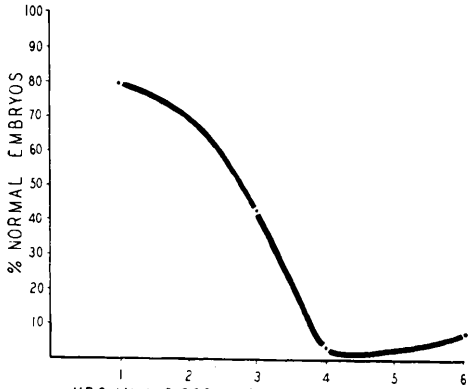
[6] Glucksohn, S., *Roux Arch. f. Entwicklungsmechanik*, 1931, **125**, 341.

[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.



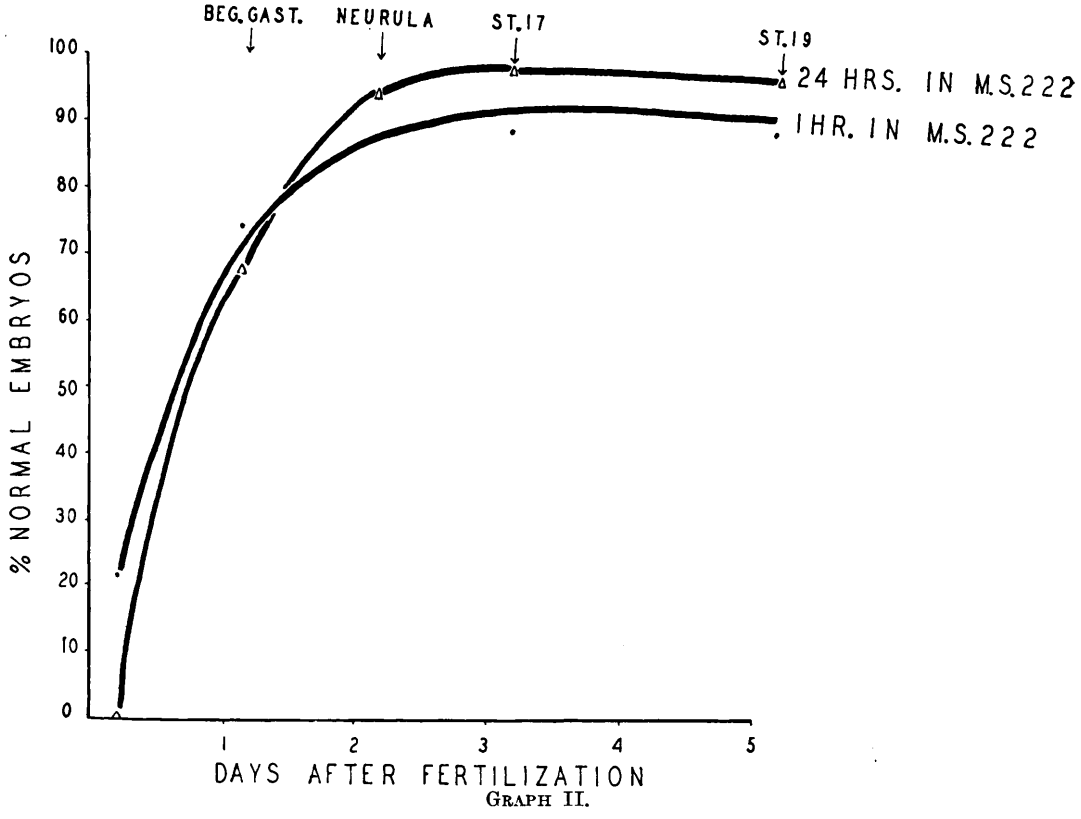
GRAPH I.

only brings out the major difference between ciliary and muscular action but also the independence of morphological and functional differentiation, in the case of muscle.

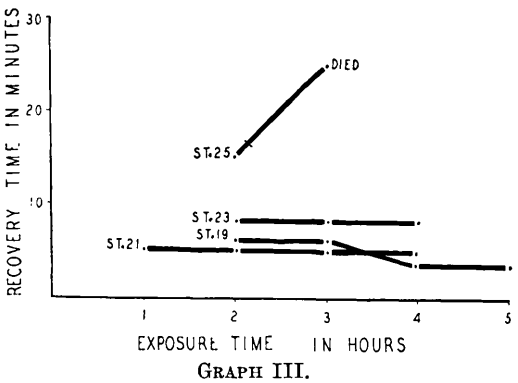
The purpose of this paper is to report some experiments which were designed to determine (1) any effect of M.S.222 on fertilization and early cleavage, (2) the speed of anesthetization at different stages of development, (3) the speed of recovery from complete anesthesia, (4) the effect on embryonic development of prolonged exposure to the anesthetic, and (5) the critically sensitive periods in early embryonic development following brief exposure to the anesthetic.

The dilution of M.S.222 used was 1/3,000 in spring water. Ovulation in the frog, *Rana pipiens*, was induced by pituitary injection and controls were kept for all experiments. Each point on Graphs I and II represents averages of 50 animals while points on Graphs III and IV represent averages of 5 readings. All experiments have been duplicated.

Graph I represents the data from fertilization of eggs in the anesthetic. A single testis was taken from each of 6 male frogs and all of them minced in 20 cc of 1/3,000 M.S.222 in spring water. The other 6 testes were minced in the same volume of pure spring water, as control material. The uterine eggs were then stripped into the sperm suspensions and were subsequently flooded with the same solution, to allow rotation. In this graph it



GRAPH II.



GRAPH III.

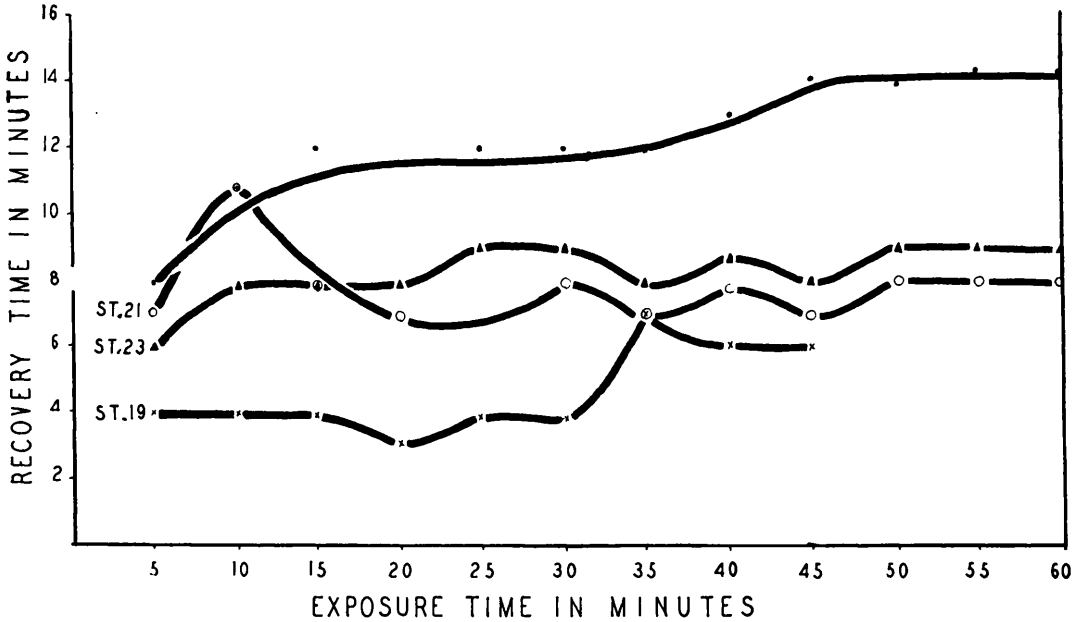
must be pointed out that the control eggs showed only 80% development so that this level must be considered maximum and normal. It appears that M.S.222 does not interfere with fertilization but that with increasing exposure to the anesthetic, prior to removal to spring water, there is a decrease in the percentage of normal development to a minimum at the 4-hour level. All of these points include the period of drastic changes that are presumed to occur in the egg immediately after fertilization.

In Graph II is found a comparison of the effect of 1-hour and 24-hour exposure to the anesthetic at different stages of development. All eggs were from a single female and were fertilized in spring water in which the controls showed 100% normal development. The two curves are much the same, indicating that the deleterious effects are more manifest when the exposure to the anesthetic is made prior to gastrulation (whether for 1 or 24 hours); slightly less so before neurulation; and negligible after neurulation. That is, after the embryo has passed through the critical periods of gastrulation and neurulation, even the 24-hour exposures were relatively harmless.

In Graph III is shown the results of exposure of later stage embryos for periods of 1 to 5 hours of anesthetization. It will be noted that stages 19, 21, and 23[10] showed recovery in 5 to 10 minutes no matter how long the exposure to M.S.222 while stage 25 died after 3 hours exposure. This exception is probably related to the fact that at this stage the operculum opens and there is a shift from external to internal gill respiration. These embryos exhibit a typical oedema and rupture of the cephalic blood vessels.

In Graph IV is shown the rate of recovery of the 4 stages of frog embryo development

[10] Rugh, R., *Experimental Embryology*, 1943, N. Y. U. Press.



M.S.222
GRAPH IV.

(stages 19, 21, 23, and 25) following various exposure periods from 5 minutes to 1 hour. The time lapse for complete muscular immobilization varied from 30 to 80 seconds, and the recovery time ranged from 4 to 14 minutes, depending upon the duration of anesthetization and the stage of development considered. Here too it will be noted that recovery time consistently was increased only in the case of stage 25.

Summary and Conclusions. (1) The anesthetic M.S.222 in 1/3,000 concentration in spring water has no effect on the motility or the fertilizing power of frog spermatozoa. (2) Frog's eggs fertilized in this anesthetic and allowed to remain for 1 hour will develop as well as do the controls. Exposure for longer periods will show an increasing percentage of abnormal embryos. (3) Embryos that show muscular activity (stage 19 and older) are completely immobilized (except for ciliary activity) within 80 seconds at ordinary laboratory temperatures, some embryos becoming quiet within 30 seconds. Recovery rate (*i.e.*, the return to muscular activity) ranges from 4 to 14 minutes, depending more on the stage of development than on the duration of anesthetization. (4) Prolonged exposure to the anesthetic produces abnormal embryos if the exposure is made prior to neurulation and kills the embryos at stage 25. This latter instance may be due to the change in the respiratory mechanism at that time. (Urodele larvæ do not show this reaction, and may be anesthetized at least up until metamorphosis.) (5) The anesthetic has no effect on ciliary action but is quick in its effect on muscular activity; it is non-toxic in the dilution of 1/3,000 in spring water for periods adequate for surgical operations; and recovery time is uniformly brief. M.S.222 has proven to be a very satisfactory anesthetic for amphibian embryos.

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Effect of Starvation and Ageing on the Acid-Soluble Phosphate Components of the Liver.

NATHAN O. KAPLAN AND DAVID M. GREENBERG.

From the Division of Biochemistry, University of California Medical School, Berkeley.

In contrast to the abundance of information on muscle phosphates, comparatively little is known regarding the acid-soluble phosphates of the liver. In connection with a tracer study of the phosphate metabolism of the liver with P^{32}, a comparison was carried out of the distribution of the acid-soluble phosphate fractions of the liver of the rat under certain extremely varied conditions of life. The analytical procedures are described elsewhere.[1]

Effect of Starvation. The distribution of the different phosphorus fractions in the livers of rats fasted for 12, 24, 48, 72, and 144 hours are recorded in Table I. For purposes of comparison, the table contains the phosphorus partition in the liver of rats who had access to food until they were sacrificed. The results in Table I, in general, are in agreement with the findings of Rapoport, Leva, and Guest.[2]

Effect of Age. That the acid-soluble phosphates of the liver changed with age was observed by Kay.[3] We considered it of value to investigate the nature of these changes more thoroughly.

For the purposes of this study, several litters of stock rats were sacrificed at different ages, beginning with 21-day-old (several days prior to weaning) and the phosphate content of the various acid-soluble fractions determined The results are given in Table II.

From Table II it will be noted that the total

[1] Kaplan, N. O., and Greenberg, D. M., *J. Biol. Chem.*, in press.

[2] Rapoport, S., Leva, E., and Guest, G. M., *J. Biol. Chem.*, 1943, **149**, 57, 65.

[3] Kay, H. D., *J. Biol. Chem.*, 1932, **99**, 85.