drop in arterial blood pressure in intact dogs, and that the dogs died within 5 days after constantly losing weight.

There is as yet no explanation as to the cause of toxicity of these polypeptides. The bleeding tendency did not cause major hemorrhages. The increased red blood cell aggregation and sedimentation rate are non-specific phenomena which are caused by all macromolecular substances which were investigated (11). The postmortem gross findings in our animals were not sufficient to explain their deaths.

The polypeptides investigated by Kenny, as well as our polymers, have multiple free negative charges whereby their oncotic efficiency is considerably enhanced due to the Donnan effect, as shown by Rosenthal *et al*(6). On the other hand, these negative charges may possibly interfere with the normal function of the cell membrane, and cause serious metabolic disturbances. It seems, therefore, desirable to extend this investigation to other, non-charged or isoelectric, water-soluble linear and multichain polyamino acids.

*Summary.* Some biological properties of 3 batches of a multichain polyamino acid, multi-(copoly-L-glutamyl-L-aspartyl) poly-L-lysine, were examined *in vivo* in dogs and in rabbits. The polymer was administered intravenously as 2 to 3% solutions of its sodium salt by one or by repeated injections. In some animals hypovolemia was produced by arterial bleeding prior to infusion. A low-molecular weight fraction of the polymer, amounting to about 10 to 20%, was excreted in the urine within a few hours. The rest of the injected polymer was not excreted in the urine, and disappeared gradually from the blood, with a constant half-concentration time of about 48 hours. As shown by its influence on blood pressure and on hematocrit, the polymer was able to combat the manifestations of hypovolemic shock. However, severe toxic manifestations appeared in many animals, consisting of diarrhea, vomiting, loss of appetite, a transient drop in blood pressure and a tendency to capillary bleeding. Progressive weakness of the animals was observed, leading to their death. The cause of toxicity of these polypeptides has not been found. Possibly their multiple negative charges may be responsible. It appears worthwhile to examine non-charged or isoelectric polyamino acids with regard to their plasma expanding properties and their toxicity.

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Received August 7, 1961 P.S.E.B.M., 1961, v108

## Preservation of Rabbit Spermatozoa: Fertility Results from Frozen Semen.* (27028)

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The influence of cold on animal spermatozoa has been studied for a considerable time. De-

* This investigation was supported during 1959-60 by CRT 5013 from Nat. Inst. Health and during 1960-61 (in part) by PHS research grant from Nat. Cancer Inst., P.H.S., and aided by grant from Am. Cancer Soc. The author acknowledges the assistance of Eugene Farrin, senior laboratory assitsant, during this project.

grees of cooling utilized in such early experiments have varied from −4°C to −269.5°C and living young have been produced from chickens, cattle, sheep and human beings from deep frozen (−75°C or lower) semen(1-8). The recovery of one fertilized rabbit ovum has been reported by Smith and Polge(9) but no verifiable reports of young being produced from frozen semen have been made. Smirnov (10) stated that he produced young but gave no experimental data to support his assertions or to allow verification by other workers.

It is the purpose of this paper to report the production of 3 litters from does inseminated with frozen rabbit semen stored 24 hours at −90°C.

*Materials and Methods.* All semen samples were collected with an artificial vagina and the semen was deposited at the mouth of the cervices with an inseminating tube made of 6 mm glass tubing, 180 mm in length, with a 30° bend 60 mm from the insertion end.

The diluter was a slight modification of the CUE extender reported by Foote and Bratton(11), having the following composition: 20 cc egg yolk, 1.160 g sodium citrate ($NA_3C_6H_5O_7 \cdot 2H_2O$), .2400 g dextrose ($C_6H_{12}O_6$), .7496 g glycine ($NH_2CH_2COOH$), .1680 g sodium bicarbonate ($NaHCO_3$), .0320 g potassium chloride (KCl), 0.0100 g citric acid, 8.0 cc glycerine ($C_3H_8O_3$) and distilled $H_2O$ to make a total volume of 100 cc.

Initial dilution of collected semen was at room temperature (20°C) using 0.15 cc raw semen per sample; the diluted semen was then cooled to 5°C during the course of one hour. Later dilutions, if made, were commenced 3 hours after collection.

The diluted semen was held at 5°C until freezing. Twenty minutes prior to freezing the diluted semen was transferred from rubber stoppered test tubes to 1/2 dram glass vials. Temperature of the vials was lowered during freezing by placing them into a −25°C isopropyl alcohol and dry ice bath. Rate of cooling was controlled, by addition of chopped dry ice, so as to result in a rate of 5°C/minute to −40°C, 6°C/minute to −52°C and 8°C/minute to −70°C. Samples were then placed directly in a mechanical freezer until thawing. Thawing was done in a 5°C water bath. After liquefaction, the vial was warmed in the palm of the hand for a few minutes and then further diluted just prior to insemination.

Litter 1. Semen was collected at 7:15 A.M. and 2 samples were diluted 1:10 with the complete diluter and placed in a refrigerator to start the cooling process. The semen was frozen 8 hours and 15 minutes later and stored at −90°C. After thawing, the 3.0 cc of diluted semen was further diluted by 1.5 cc of a sterile commercial bovine semen diluter (Nasco, Inc., Fort Atkinson, Wisc.) to stimulate motility, and then inseminated.

Litter 2. Semen was collected at 5:45 A.M. and 2 samples were used. One was diluted 1:10 with the complete diluter and the other was diluted 1:5 with a non-glycerolated diluter. Three hours and 45 minutes later an equal quantity of a glycerolated diluter containing 16% glycerine was added in the manner of Bratton *et al.*(12) except that a 15 minute interval was used instead of a 20 minute interval. Freezing commenced 5 hours after the first glycerine addition was made and the semen was stored frozen for 24 hours. To the 3.0 cc of thawed diluted semen, 1.5 cc of sterile diluter was added prior to insemination.

Litter 3. A semen collection was made at 6:55 A.M. and one sample (0.15 cc) was diluted 1:5 with the non-glycerolated diluter. Glycerol addition commenced 3 hours after collection in the same manner as in Litter 2 with freezing commencing 5 hours later. 1.5 cc sterile diluter was added to the 1.5 cc diluted, thawed semen prior to insemination.

*Results and Discussion.* A total of 6 does conceived out of 22 inseminations, based on palpation at 12 and 19 days. Three of these produced live young (litters 1, 2 and 3 with 3. 3 and 7 young born on May 14th, 16th and 22nd, 1961 respectively) and the remaining 3 pregnancies resulted in one doe resorbing at midpregnancy, and the other 2 each having one young which was carried *in utero* to 3 days over the normal 31 day gestation period, and then expelled as slightly resorbed fetuses. One of the 2 does which carried one young to 34 days was bred with semen stored 13 days at −90°C.

Ovulation was stimulated in all cases by use of a vasectomized male 15-30 minutes prior to insemination. All vasectomized males were

checked by semen collections to insure complete vasectomy and litters 2 and 3 were positively identifiable based on color inheritance as being only from frozen semen.

Polge and Rowson(13) and Bratton *et al.*(12) have shown that storage time is not a critical factor for bovine semen preservation, thereby implying that the same may be true for the rabbit. Detailed experimentation is needed, however, to verify this for the rabbit.

*Summary.* Three live litters, totaling 13 rabbits, have been produced for the first time from frozen rabbit semen stored 24 hours at −90°C. The diluter used was a modified CUE diluter containing 8% glycerine. Vasectomized males were used to induce ovulation and genetic proof was presented to show that the young could not have come from the vasectomized males.

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Received August 9, 1961 P.S.E.B.M., 1961, v108

# Biosynthesis of Cholesterol XI. Biosynthesis of Cholesterol and Fatty Acids in Pregnant Rats.* (27029)

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The formal parallelism of the intrauterine growth of the embryo to tumor growth in the body of the host was the reason why the results obtained in this laboratory in a study of the biosynthesis of cholesterol and fatty acids in tumor bearing animals(1) suggested a similar study of the biosynthesis of cholesterol and fatty acids during pregnancy.

*Methods.* Virgin female Sprague-Dawley rats weighing about 270 g each were used. They were mated within 6 hours during the same day. All those animals which were allowed to carry to term gave birth to their young 21 days after mating. At the intervals given below for each experiment, a group of rats was injected with 0.5 mc of sodium acetate-1-$C^{14}$ per kg body weight and 2 animals were killed 10, 20, 40, 80 and 240 minutes respectively after the injection. They were then dissected to obtain livers, gastrointestinal tracts, carcasses and fetuses. The tissues, after being weighed were hydrolyzed overnight on a steam bath in 30% KOH. After acidification, cholesterol and fatty acids were extracted with pentane. Purification of cholesterol and counting of samples were carried out as described before(2). Cholesterol determinations in serum were made with the method of Abell et al.(3) using the blood obtained by heart puncture from 4 animals for each determination at 6, 12, 20, 35 and 56 days after mating.

*Experiments.* Exp. 1: Ten non-mated rats were injected on the mating day with sodium acetate-1-$C^{14}$ as described to serve as controls.

2: Ten pregnant rats were injected 6 days after mating. Because of the impossibility of separating the placenta or fetuses, whole uteri were used.

3: Nine pregnant rats were injected 12 days

* Supported by Grants from Am. Cancer Soc. and Nat. Science Foundation.