An amount of bleaching of the dye solutions occurs which is detectable spectrophotometrically; the quantities are indicated in Table I. We have been forced to conclude that the products of this bleaching, and not peroxide are responsible for at least the greater part of the increased hemolytic properties of our solutions. Further experiments are in progress.

7415 P

Observations on the Treatment of Infertile Rabbits with Antuitrin "S".

PAUL D. ROSAHN, HARRY S. N. GREENE AND C. K. HU.

From the Laboratories of The Rockefeller Institute for Medical Research.

Prolonged experience with a large breeding colony of rabbits has revealed that a small proportion of sexually mature females repeatedly fail to become pregnant after being served by known fertile bucks. In general, such does are in excellent physical condition and show no external evidence of disease, although a few have a tendency to obesity characterized by a shoulder girdle adiposity and an abnormal accumulation of fat in the abdomen. Recently, certain of these persistently infertile does carried genetic factors which were under investigation, and progeny from them were desired in order to carry on the genetic studies. Accordingly, they were submitted to different therapeutic procedures, with a view to developing a method which would render them fertile. Since all the animals were of value because of their genetic constitution, operative interference and experiments hazardous to life were of necessity avoided. The present report is concerned with one of the methods employed in the treatment of these does.

In the rabbit, ovulation occurs about 10 hours after copulation. It was thought that the threshold necessary to induce ovulation in refractory females was higher than the stimulus afforded by the act of coitus. A simple method of augmenting the copulation stimulus lay in the observation of Friedman[1] that the parenteral administration of human pregnancy urine induces ovulation in the rabbit. The method employed in the present investigation was to inject a single dose of 40 Rat Units per kilo body weight* of Antuitrin "S"

[1] Friedman, M. H., *Am. J. Physiol.*, 1929, **90**, 617.

* This dose was suggested by the work of Snyder, F. F., *Bull. Johns Hopkins Hosp.*, 1934, **54**, 1.

into the marginal ear vein of the doe. The time of mating with a known fertile buck with reference to the injection varied from 22 hours before to 24 hours after the administration of Antuitrin "S". After a lapse of 10 days, when clinical examination determined the existence of a non-pregnant state, the procedure was repeated with a few does. The investigation was begun on January 18 and continued to March 19, 1934. The control period of matings with which the experimental results were compared began in the middle of September, 1933, and continued up to the time the animal was treated, thus ranging from 4 to 6 months. It was during this interval that repeated matings established the infertility of the doe and determined her availability for this study.

Forty-three trials were made on 27 rabbits, each trial consisting of the administration of Antuitrin "S" plus service by a fertile buck. Six deaths to be discussed later followed the administration of the pregnancy urine principle. These occurred in 4 animals being observed for the first time and in 2 animals each of which had been tested on one previous occasion. The results are, therefore, based on 37 trials with 23 animals.

Nine pregnancies and 28 infertile matings resulted from the 37 trials. During the control period, the 23 animals in the group had been served on 122 separate occasions with 9 pregnancies resulting. The occurrence of pregnancy in relation to the Antuitrin "S" treatment was significantly more frequent than the incidence of pregnancy during the control untreated period ($\chi^2 = 8.05$, $P = 0.01-$, significant). The series was divided into 2 groups depending on the relation of the Antuitrin "S" treatment to the time of mating. In 9 trials the drug was given from 20-24 hours before the mating—no pregnancies resulted. Twenty-eight injections within a 2-hour period before to 22 hours after mating resulted in 9 pregnancies. Thus more pregnancies were noted when the Antuitrin "S" was given from 2 hours before to 22 hours after mating than when it was injected 20-24 hours before ($\chi^2 = 3.85$, $P = 0.05-$, probably significant). Nine pregnancies occurred in the group of 28 trials on 16 animals receiving the drug within 2 hours before to 22 hours after service. These 16 animals had been mated 85 times during the control period and only 2 pregnancies resulted. The relation of pregnancy and treatment in this group was very highly significant ($\chi^2 = 21.56$, $P = 0.01-$, significant). In this treated group 9 of the 16 animals became pregnant after an average of 1.7 matings per animal, whereas in the untreated control period pregnancy was noted twice in only one of these 16 rabbits following a mean of 5.3

matings per animal ($\chi^2 = 9.32$, $P = 0.01-$, significant). One persistently infertile doe did not become pregnant when mated in conjunction with treatment. The next mating 10 days later without treatment resulted in a pregnancy, the first in 6 attempts, and this was followed by a second pregnancy also without treatment. In 2 instances, does which were non-pregnant after treatment, became pregnant following a subsequent administration of the drug plus service.

Five of the 6 deaths took place from 2-18 hours after the administration of Antuitrin "S" and were definitely associated with the treatment. The sixth death occurred 7 days after the injection and may or may not be related to the drug. All of these animals showed evidence of an acute physiological upset. Congestion and hemorrhage of the subcutaneous vessels particularly in the region of the mammary glands, and congestion and hemorrhage of the thymus, vagina and uterus were frequent findings. The exact cause of these deaths is not known at present, but the common feature of their occurrence in abnormally obese animals suggests a violent endocrine and vasomotor crisis precipitated by the drug.

The results suggest that one factor involved in the sterility of certain does is their failure to ovulate after coitus, and that this failure may be remedied by the administration of pregnancy urine principle. It is possible that repeated injections and doses larger than 40 Rat Units per kilo might cause ovulation in those does which were refractory to the treatment described herein. There is some evidence to support the view that the stimulating effect of Antuitrin "S" may lower the ovulation threshold, so that a subsequent mating after the lapse of many days and even weeks, might have a fertile result without its adjunct at the time of the succeeding matings.

7416 P

Observations on Vitamin Treatment of Reproductive Abnormalities in the Rabbit.

C. K. HU, PAUL D. ROSAHN AND HARRY S. N. GREENE.

From the Laboratories of The Rockefeller Institute for Medical Research.

The reproductive activities of certain rabbits in our breeding colony have been observed on repeated occasions to be abnormal. These abnormalities may be classified, for present consideration, in 3