for 5 passages in eggs, acidophilic small granules that were accumulated around the nucleolus was the only change observed. In the yolk sacs of eggs inoculated with saline, no changes were seen (Fig. 2).

Brains from guinea pigs inoculated intracerebrally with the rabbit brain virus and virus that had been transferred for 50 passages in eggs were fixed in Zenker's or formalin fixative and stained as for the egg material. In some brains from guinea pigs inoculated either with virus passed in rabbits or virus transferred for 50 serial passages in eggs, slight congestion was the only change observed, while in others, leucocytic infiltration and marked congestion of the blood vessels of the meninges and the superficial part of the brain were seen. Most of the animals showed the superficial nerve cells in different stages of degeneration. Intranuclear inclusions similar to those observed in the cells of yolk sacs from infected eggs were found in some nerve cells (Fig. 3 and 4).

Summary. Using the yolk sac method, a strain of pseudorabies virus has been cultivated in eggs for 50 serial transfers. That the virus multiplied in the yolk sac was shown in 4 ways: (1) suspensions of yolk sacs from infected eggs that had been transferred either for 5, 12, 26, 40 or 50 passages produced disease in rabbits, guinea pigs and mice; (2) the effects in animals of virus that had been transferred for 50 serial passages was neutralized by antiserum that neutralized virus passed in rabbits; (3) the yolk sac contained as much or more virus than either the chorioallantoic membranes, chorioallantoic fluid or embryos; (4) the virus produced characteristic intranuclear inclusions in the yolk sac cells like those seen in cells from infected animals.

With continued transfer of pseudorabies virus in eggs, embryos died 40 hours after inoculation while in the initial passage only 60% of the embryos died after 3 days. The capacity to invade certain parts of the egg such as the chorioallantoic fluids and embryos increased and more extensive changes were produced in the embryo. Continued passage also altered the effects on rabbits, guinea pigs and mice. The period of time from inoculation until death of animals lengthened, the local pruritus produced by subcutaneous inoculation of virus passed in rabbits no longer occurred and infected animals lived longer after showing signs of illness.

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17212. Antagonism of Choline and Heparin *In vivo* and *In vitro*.*

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Cabezas and Honorato[1] reported that heparin was inactivated by choline chloride. Various dilutions of heparin, choline and combinations of the 2 were added to fresh and 24-hour preserved plasma. The influence upon the prothrombin time was determined by the one stage Quick method.[2] These investigators did not draw final conclusions as to the mechanism of this effect, but suggested that there may be a chemical union of the choline with heparin, seroalbumin or albumin X.[3]

The annulation of heparin with prota-

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mine or toluidine blue is well established.[4-6] These latter compounds are complex molecules whereas choline is a simple molecule. $(CH_3)_3 N(OH)-CH_2-CH_2OH$. In view of the recent work by Allen, Jacobson, Smith *et al.*, on the hemorrhagic syndrome produced by exposure to heavy doses of X-irradiation or nitrogen mustard,[7,8] the inactivation of heparin by choline becomes of considerable physiological and clinical interest. They have shown that this syndrome is due to an increase in the heparin or a heparin-like compound in the blood. Through 2 approaches *in vitro* and one approach *in vivo*, we have attempted to elucidate this problem.

Influence of choline and heparin upon the prothrombin time. A portion of Cabezas and Honorato's work was repeated as accurately as possible. The prothrombin time, Quick method,[2] was carried out on fresh whole human plasma. Choline chloride (2% and 4%) (Merck) in physiological saline was used in various volumes. Commercial heparin, 10 mg/cc (Roche-Organon) was diluted with physiological saline so that 0.025 cc equaled approximately 0.4 International Units and 0.025 cc equaled approximately 0.2 International Units. The commercial heparin contains approximately 110 International Units per mg. Rabbit brain thromboplastin (Maltine Co.) was used. The choline-heparin mixtures were not incubated.

Results. The prothrombin times on the control plasmas averaged 14.5 seconds; 0.2 U heparin added to the plasma showed an increase in the prothrombin time, averaging 4.9 seconds; 0.4 U heparin increased the prothrombin time an average of 13.7 seconds. Fifty γ, 2% choline, increased the prothrombin time an average of 1.0 seconds; 100 γ, 2% choline, increased the prothrombin time an average of 3.1 seconds; 100 γ, 4% choline, increased the prothrombin time an average of 2.9 seconds. Mixtures of heparin and choline in various concentrations and volumes added to the plasma always produced an increase in the prothrombin time over that of the heparin alone.

Interpretation. Our data illustrated no inactivation of the heparin by the choline. The slight increase in prothrombin time demonstrates the *in vitro* anticoagulant influence of choline. This confirms the observations of Zunz and LaBarre.[9]

When one analyzes Cabezas and Honorato's results, it is observed that the control prothrombin times were prolonged in most experiments. This is evidence of relatively inactive thromboplastin.

Absence of a chemical union of heparin and choline as measured by spectrophotometric determination. By the use of the Beckman spectrophotometer the absorption spectrum of a 0.1% solution of powdered heparin (Connaught 110 U/mg) dissolved in distilled water was determined. The peak of the absorption curve was found to be 265 mμ. Choline chloride in a 0.1% solution has no typical absorption bands. The two solutions were mixed in equal parts and the absorption curve was determined immediately, and after 6 hours of incubation.

Results. As may be seen in Fig. 1, there was no change in the absorption spectrum after heparin and choline were mixed.

Interpretation. If choline were combined with heparin there would be a definite shift in the spectrum of the choline-heparin mixture from that of the heparin alone. This demonstrates quite conclusively that heparin and choline do not enter into a chemical combination.

In vivo influence of heparin-choline on coagulation and prothrombin time. A series of experiments were designed to give *in vivo* evidence concerning the action of these substances on the coagulation mechanism. Three rabbits, each weighing 2.0 kg, were injected

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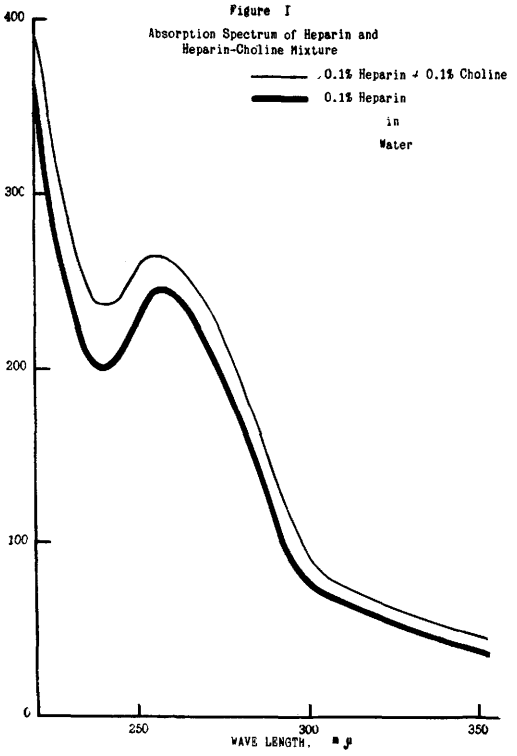

intravenously in an ear vein weekly with solutions of heparin and choline in the following ways:

1) 100 IU/kg heparin;
2) 10 mg/kg 2% choline chloride in saline;
3) 100 U/kg heparin followed immediately by 10 mg/kg choline;
4) 10 mg/kg choline followed in 5 min. by 100 U/kg heparin;
5) 100 U/kg heparin followed in 5 min. by 10 mg/kg of choline.

Blood was obtained by cardiocentesis with a 22 gauge needle. A specimen was drawn prior to and 10, 20, 30 and 60 minutes after injection. Coagulation times were done by a modification of the Lee-White method.[10] Blood in the amount of 0.5 cc was added to a 10 by 75 mm glass tube which had been rinsed with saline. The tube was tilted every 30 seconds and was kept at a temperature from 32 to 35°C. The end point was taken as the time the tube could be tilted vertically and the clot was solid. The prothrombin time was done by the one stage Quick method on whole plasma, and 0.9 cc of blood was added to 0.1 cc of 0.1 M sodium oxalate.

Results. Normal coagulation time varied between 3½ and 14 minutes. The choline alone had no remarkable effect on the blood coagulation other than possibly slightly accelerating it. Heparin alone, and heparin and choline in the various combinations, produced similar results.

The normal prothrombin times varied between 5.7 and 7.8 seconds and were not affected by the choline, whereas, they were slightly prolonged from 0.7 to 2.4 seconds after heparin alone, and 18.5 seconds after the heparin-choline injections. All prothrombin times were done in duplicate. If more than one second difference was obtained a third test was performed.

Interpretation. By means of the coagulation time and prothrombin time *in vivo,* choline has been shown to have no antagonistic influence on the heparin effect. One interesting observation is that choline alone consistently accelerated coagulation. This group of rabbits is too small, however, to draw definite conclusions as to the effect of choline on coagulation. Therefore, this is being studied further.

Summary. The previously reported antagonism of choline and heparin was studied *in vitro* and *in vivo.* When choline and heparin were mixed in whole plasma and the prothrombin time determined, the choline had no effect on the action of heparin. Choline alone slightly prolonged the prothrombin time. Choline and heparin do not combine chemically as shown by spectrophotometric determination. *In vivo,* choline has no effect on the action of heparin.

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