not contraindicated in patients with coronary disease.

Summary. The intravenous administration of 0.5 mg of dihydroergocornine to 24 patients with left ventricular hypertrophy caused no electrocardiographic changes indicative of coronary vasoconstriction. Although 6 of the 24 patients suffered from anginal pain and many more certainly had coronary disease, no angina pectoris followed the injection.

This particular alkaloid of ergot seems to be a safe drug in patients with coronary artery disease.

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17211. Cultivation of Pseudorabies Virus in the Yolk Sac of the Developing Chick Embryo.

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Successful cultivation of pseudorabies on the chorioallantoic membrane of embryonated eggs has been achieved by a number of workers,[1-6] several of whom propagated the virus in serial transfer for more than 50 passages. They noted that the titer of virus increased with continued passage, ability to kill embryos was gained and a lengthened incubation period for laboratory animals was acquired. The virus, however, invariably produced fatal infection. Although no reports were found that related to the cultivation of pseudorabies virus by the yolk sac method of Cox,[7] the attenuation of rinderpest by Jenkins and Shope[8] and of Newcastle virus by Beach[9] prompted the hope that cultivation in this manner might modify pseudorabies virus also. In both the rinderpest and Newcastle studies attention was centered on virus obtained from fluids, chorioallantoic membranes and embryos while titers attained in yolk sacs were not mentioned specifically. Indeed, other than for rickettsial and psittacoid agents, no information could be found that covered the growth of the smaller viruses in the yolk sac. In the work that follows attention was focused on growth of pseudorabies in the yolk sac membranes and the consequent effects on the virus.

Technic. The pseudorabies virus used throughout this work was a Hungarian strain sent to us through the courtesy of Dr. R. E. Shope in an infected rabbit brain preserved in glycerol. Part of the infected rabbit brain after washing was ground with sterile saline to make a homogeneous suspension and then 0.2 cc was inoculated intracerebrally into a rabbit. Two days later and immediately after death of the animal with typical symptoms of the disease, the brain was removed aseptically, weighed and ground in saline with a glass grinder to make a 10% suspension. The suspension was distributed in vials, sealed, quickly frozen and stored under dry ice refrigeration. Virus stored under such conditions was used to inoculate eggs in the initial passage. In subsequent passages 10% suspensions of fresh yolk sacs, prepared as for the rabbit brain, were used except in the first

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TABLE I.
Results in Experimental Animals Inoculated Intracerebrally with Various Dilutions of Pseudorabies Virus Cultivated in the Yolk Sac of Chick Embryos.

Test animals*	Yolk sac passage	Dilutions used†				
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Rabbits	0	—	1/1	2/3	0/2	—
	5	—	1/1	1/1	1/1	0/2
	12	—	1/1	1/1	1/1	0/1
	26	—	—	1/1	0/1	0/1
	40	—	2/2	1/1	0/1	—
	50	—	1/1	1/1	1/1	0/1
Guinea pigs	0	1/1	2/2	0/2	0/1	—
	5	1/1	3/3	3/3	0/2	—
	12	1/1	2/2	4/4	2/2	0/1
	26	1/1	1/1	1/1	0/1	0/1
	40	1/1	1/1	1/1	0/1	—
	50	1/1	1/1	1/1	1/1	0/1
Mice	0	3/3	12/12	2/6	0/3	—
	5	3/3	9/9	6/6	0/3	—
	12	3/3	6/6	9/9	5/6	1/3
	26	3/3	3/3	3/3	0/3	0/3
	40	—	3/3	3/3	0/3	—
	50	—	3/3	3/3	2/3	0/3

* .2 cc of the virus dilution inoculated in each rabbit and guinea pig and .05 cc in each mouse.

† Denominator indicates number of animals used; numerator indicates animals that showed pseudorabies.

TABLE II.
Results in Experimental Animals Inoculated Subcutaneously with Various Dilutions of Pseudorabies Virus Cultivated in the Yolk Sac of Chick Embryos.

Test animals*	Yolk sac passage	Dilutions used†							
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Rabbits	0	—	—	—	2/2	2/2	0/1	—	—
	5	—	—	—	1/1	1/1	1/1	0/1	—
	12	—	—	—	1/1	1/1	1/1	1/1	0/1
	26	—	—	—	—	1/1	0/1	0/1	—
	40	—	—	1/1	1/1	1/1	0/1	—	—
	50	—	—	1/1	1/1	1/1	0/1	—	—
Guinea pigs	0	1/1	3/3	2/3	0/1	—	—	—	—
	5	—	2/2	3/3	3/3	0/1	—	—	—
	12	1/1	1/1	2/2	3/3	2/2	0/1	—	—
	26	1/1	1/1	1/1	0/1	0/1	0/1	0/1	—
	40	2/2	1/1	1/1	0/1	0/1	—	—	—
	50	2/2	1/1	1/1	0/1	0/1	—	—	—
Mice	0	—	3/3	2/3	0/6	0/3	0/3	—	—
	5	—	6/6	3/3	6/6	0/3	0/3	—	—
	12	—	3/3	3/3	6/6	0/3	0/3	—	—
	26	—	—	3/3	1/3	0/3	0/3	0/3	—
	40	3/3	3/3	3/3	0/3	—	—	—	—
	50	—	6/6	3/3	0/3	—	—	—	—

* 1 cc of the virus dilution inoculated in each rabbit and guinea pig and 0.5 cc in each mouse.

† Denominator indicates number of animals used; numerator indicates animals that showed pseudorabies.

few passages where this material was stored for 48 hours at 34°F. This condition of storage was found not to affect the titer of virus. In tests for sterility, samples from all inocula were placed on blood agar plates and blood agar slants. All showed no growth.

TABLE III.
Distribution of Pseudorabies Virus in Eggs Inoculated into Yolk Sac.

Material used	Yolk sac passage	Dilutions				
		10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Yolk sac	1	0	+	+	+	0
	14	0	+	+	+	—
	50	0	0	+	+	—
Chorioallantoic membrane	1	0	+	+	—	0
	14	0	+	+	—	0
	50	0	+	+	—	—
Chorioallantoic fluid	1	+	—	—	0	0
	14	0	+	+	+	—
	50	0	0	+	—	—
Embryo	1	+	—	—	0	0
	14	0	+	+	—	0
	50	0	+	+	—	—

+ Virus shown to be present when inoculated intracerebrally into guinea pigs.
—Virus not present.
0 Not done.

In attempts to cultivate pseudorabies virus in the yolk sac, 9 fertile hens' eggs that had been embryonated for 6 days were inoculated in each passage and 3 eggs were left uninoculated as a test of incubator conditions. After inoculation, all eggs were incubated at 37°C and thereafter candled twice daily to record any changes in the embryos. Immediately after death of the embryos, the amount of virus was determined in the following manner. Tenfold dilutions were made from culturally sterile 10% suspensions in chilled saline and held in iced water from the time of preparation to completion of inoculations. From each dilution, 0.2 cc was inoculated intracerebrally into each of 1 or more rabbits and guinea pigs, 1 cc subcutaneously into each of 1 or more rabbits or guinea pigs, 0.05 cc intracerebrally into each of a group of 3 or 12 mice, and 0.5 cc subcutaneously into each of a group of 3 or 6 mice. Animals were observed twice daily and signs of illness recorded. In animals that showed no signs of illness, it was found that the first inoculation had conferred no immunity.

Transfer of virus in serial passage. After 5, 12, 26, 40 and 50 serial passages, tests were made for the presence and content of virus in the yolk sac. These results in comparison with the virus passed in rabbits are shown in Tables I and II.

As can be seen in Tables I and II, it appeared that the virus was maintained in the yolk sac in serial transfer for 50 passages and that the titer remained unchanged or perhaps increased. As tested by intracerebral inoculation in mice, serum that neutralized a hundred infective doses of the virus passed in rabbits likewise neutralized a similar amount of virus transferred in eggs for 50 passages. This result permitted the conclusion that the death produced in rabbits, guinea pigs and mice with suspensions of yolk sac from eggs after 50 serial transfers was due to pseudorabies virus and not another agent encountered in passage.

Distribution of virus in the egg. After showing that pseudorabies virus infected yolk sacs, it became of interest to determine further distribution of the virus in eggs inoculated in this manner. Each of a group of 12 eggs was inoculated with a 10% suspension of brains from infected rabbits, another with a 10% suspension of yolk sacs from infected eggs that had been transferred serially for 13 passages, and a third group with a 10% suspension of yolk sacs from infected eggs transferred for 49 passages. Eggs inoculated with virus passed in rabbits were harvested 3 days after inoculation, while those in the serial passages were harvested after 40 hours. From these eggs chorioallantoic fluids, yolk sacs, embryos and chorioallantoic membranes were obtained separately, and with the excep-

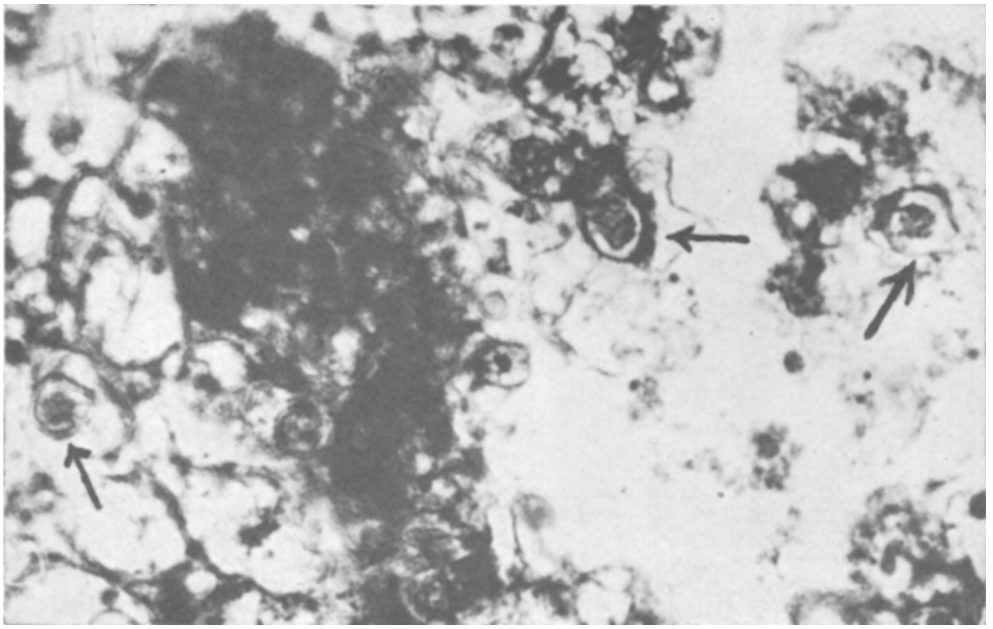

FIG. 1.
Yolk sac from an egg inoculated with virus transferred for 50 passages. Arrow indicates intranuclear inclusions.

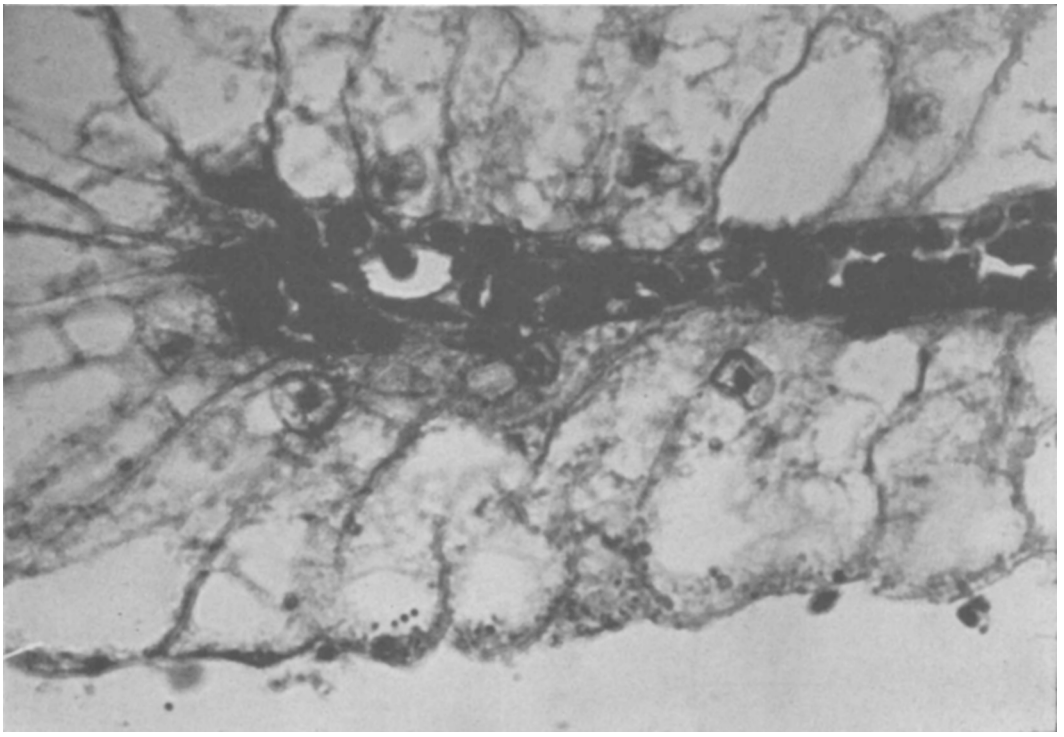

FIG. 2.
Yolk sac from an egg inoculated with saline, showing normal appearance of nuclei of yolk sac cells.

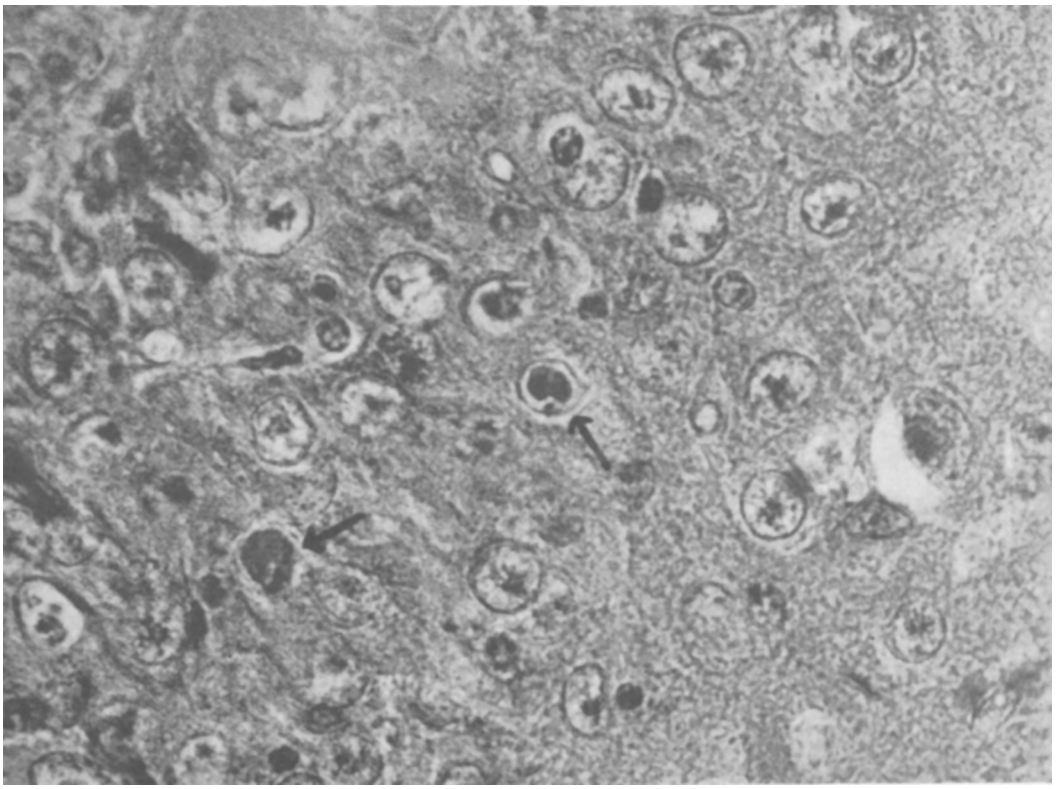

FIG. 3.
Brain from infected guinea pig inoculated intracerebrally with virus passed in rabbits. Arrow indicates inclusions in nuclei.

tion of chorioallantoic fluids, washed in 3 changes of saline, triturated and 10% suspensions made. Tenfold dilutions then were prepared from each suspension and tested in guinea pigs by intracerebral inoculations. The results are shown in Table III.

In Table III, it can be seen that the yolk sac always contained as much or more virus than other parts of infected eggs.

Effect of virus transferred in eggs on animals. In the first passage of pseudorabies virus into eggs, 60% of the embryos died 3 days after inoculation, in the 5th passage 80% of the embryos died within 48 hours, and in the 12th and subsequent passages all embryos died within a period of 40 hours.

In the rabbits, guinea pigs and mice inoculated both intracerebrally and subcutaneously with virus passed in rabbits and transferred 5, 12, 40 and 50 passages, comparisons were made of the effects produced by 10 to 100 lethal doses of virus. It was found that with continued serial transfer of virus in eggs, the interval of time between inoculation and death lengthened and animals survived longer after showing visible signs of illness. Moreover, a marked change in the signs of experimental disease was observed in inoculated animals. When rabbits were inoculated with virus passed in rabbits subcutaneously, characteristic skin signs and lesions in the form of an intense pruritus at the site of inoculation invariably developed. The skin became denuded of hair, abraded and infiltrated with serosanguinous fluid. Virus transferred in the yolk sac for 5, for 12, for 26 and for 40 passages produced similar signs, while animals that received virus transferred for 50 passages failed to develop signs or lesions in the skin. Instead, leg weakness developed 2 to 4 days before they died. Guinea pigs showed changes similar to rabbits except that the characteristic signs and lesions disappeared when the virus had been transferred for 40

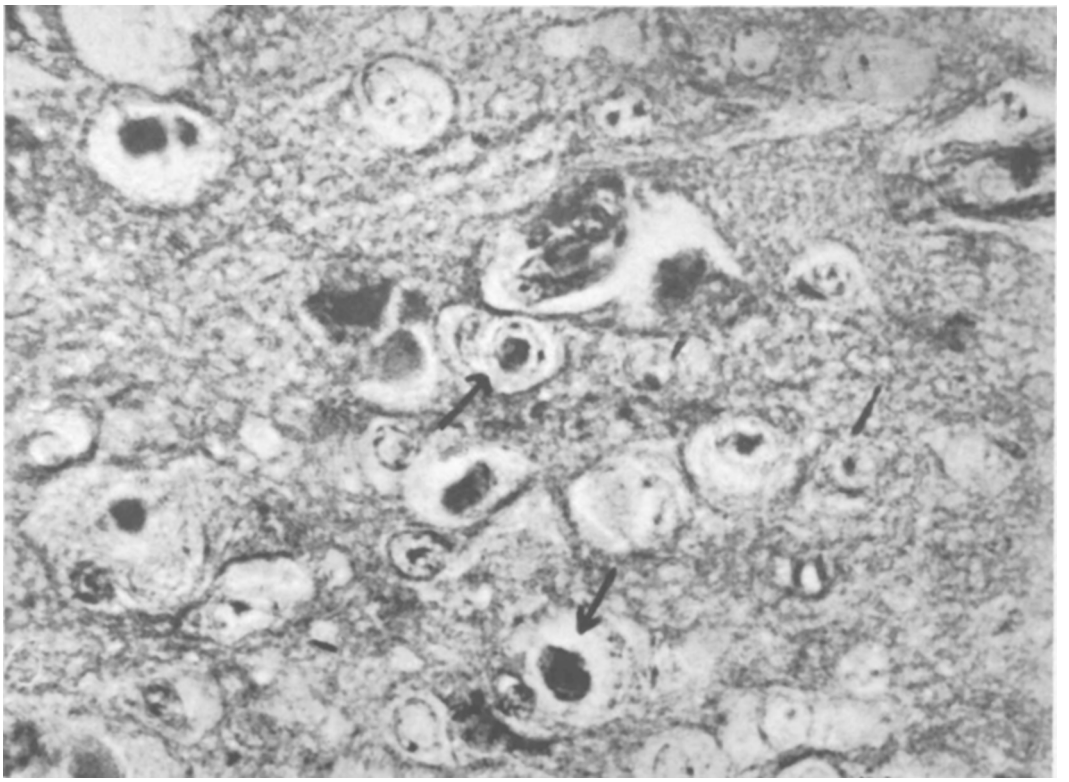

FIG. 4.
Brain from guinea pig inoculated intracerebrally with virus transferred for 50 serial passages in eggs. Arrow indicates inclusions.

passages in eggs. The animals then died with signs simulating those in guinea pigs inoculated intracerebrally. Mice that were inoculated subcutaneously showed paralytic-like signs when given virus transferred for 40 passages in eggs. Pruritus was not seen in mice even when inoculated with virus passed in rabbits.

Pathology. Embryos from the initial passage and each subsequent one were examined for lesions. In the first few passages, hemorrhages in the skin were seen over the brain region, but later hemorrhages extended over the entire surface of the embryos.

Brains of embryos from eggs inoculated with virus transferred for either 15 or 50 serial passages in eggs were prepared in Zenker's fixative and stained with hematoxylin and eosin and by Wilhite's method. Examination showed congestion of the superficial blood vessels with leucocytic infiltration throughout the brain. The neurons and neuroglial cells showed various stages of degeneration. Some of the mesothelial cells of the membranes covering the brain showed intranuclear inclusions in the form of homogeneous acidophilic material filling part or all of the nucleus. In brains of embryos from eggs inoculated with either rabbit brain virus or virus transferred for 5 passages, these changes were less pronounced and in some, only a slight congestion of the superficial blood vessels was found. Brains of embryos from eggs inoculated with saline showed no changes.

The most important changes seen in the cells of yolk sacs from eggs inoculated with virus transferred 50 passages were intranuclear inclusions (Fig. 1) that appeared as acidophilic homogeneous material filling most of the nucleus when completely formed. Margination of the chromatin along the inner surface of the nuclear membrane was seen. In the yolk sac cells of eggs inoculated with virus passed in rabbits and virus transferred

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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