Further, the response to plasma containing urea was transient with a reverse response when control plasma perfusion was resumed. It would seem that simple movement of water across the capillary wall is not a primary factor in the osmotic response. Although it would appear that cell membranes are involved in the response, the mechanism remains to be elucidated.

Another general mechanism suggested for autoregulation in response to arterial and venous pressure changes is that of compression of the resistance vessels by tissue pressure secondary to transcapillary water movement. The osmotic response would fit this model in general. However, the osmotic response is complete in 1–2 min and is quite stable. For the tissue-pressure, vascular compression model to reach equilibrium so soon would require that the involved extracapillary space be very small.

It is also possible that the response is simply the result of swelling by hydration or shrinking by dehydration of the endothelium or some other part of the vascular bed, swelling being at least partly into the lumen of the vessels, thereby decreasing luminal cross section and increasing resistance to flow. This hypothesis seems the least favorable of those suggested but evidence to support or refute it awaits further study.

Summary. The gastrocnemious–plantaris muscle group of the dog was pump-perfused at constant flow with fresh plasma from a donor dog. When the osmolality of the perfusing plasma was increased by adding sodium chloride, glucose, sucrose, mannitol, dextran, or urea, the resistance to flow through the muscle decreased. The decrease in resistance was proportional to the increase in plasma osmolality. When distilled water was added to the plasma to decrease the osmolality, the resistance to flow through the muscle increased in proportion to the decrease in osmolality. The response to most test substances persisted for at least 20 min. These data suggest a mechanism regulating blood flow in resting muscle that is sensitive to osmolar concentration differences.

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Purification of a Horse Placental Inhibitor to Hemagglutination by H-1 or HB Viruses* (32999)

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The H-viruses are a group of minute DNA agents, approximately 200 Å in diameter, that have been found associated with rapidly proliferating tissues such as tumors and embryos (1). H-1 and HB have been isolated from human tissues, H-3 from a transplantable human tumor carried in rats, and RV from rat tumors only. All of the agents, which can be distinguished from one another serologically and by their hemagglutination patterns, produce a "mongoloid-type" deformity in newborn hamsters. In 1964, Toolan (2) reported that fluid expressed from human placentas inhibited the hemagglutination (HA) of guinea pig red cells by H-1 and HB viruses but did not affect HA by the closely related agents, H-3 or RV. Human maternal or fetal sera, on the other hand, did not prevent HA by any of these viruses.

Recent studies (3,4) have shown that the inhibitor in the human placental fluids is apparently a glycoprotein that sediments as a

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TABLE I. Activity of Various Fractions Obtained during Purification of an H-1 Virus Hemagglutination Inhibitor from Horse Placentas.[a]

	HA-I titer	Total units of HA-I activity	Total protein	Purification factor
Orig. placental fluid	640[b]	14.4×10^{4}[c]	8707	1
Kaolin absorbed fluid	80	14.4×10^{4}	3240	2.7
Centrifugation pellet	2560	18.1×10^{4}	437	25
Active column eluate	5120	17.9×10^{4}	7.9	1360
Active sucrose fractions	10240	10.2×10^{4}	2.3	2660

[a] Each placental fluid was purified separately as soon as the placenta was received. The above table represents the results obtained with a single placenta. All 8 placentas tested gave similar results.

[b] Guinea pig cells were employed for these procedures. Results are expressed as the highest dilution that completely inhibited hemagglutination (HA-I titer).

[c] Titer times ml of material.

macroglobulin whose biological activity can be destroyed by neuraminidase or receptor destroying enzyme as well as by trypsin, chymotrypsin, or papain. Our current studies were undertaken to determine whether horse placental fluids also contain an inhibitor for hemagglutination by any of the H-viruses. Preliminary work showed that horse placental fluids, even when absorbed with kaolin to remove non-specific inhibitors (5, 6, 7), also prevent HA of H-1 or HB viruses with guinea pig red blood cells while maternal horse or foal sera are ineffective. Again, the placental fluids have no effect on the hemagglutination of H-3 or RV viruses. As in the human, the inhibitor substance is apparently a product of the placenta itself. The present report describes the isolation and characterization of the horse placental inhibitor.

Materials and Methods. Horse placental fluids were obtained from fresh unwashed horse placentas by allowing liquids of the placental mass to leak out into a containing vessel. Since these placentas were not handled originally with aseptic precautions, the fluids were filtered through Selas-03 filters after kaolin absorption and then tested for bacterial contamination. The fluids so treated and used for the purification procedures herein described, were free of bacteria as determined by thioglycollate broth and blood agar slant cultures. Approximately 250 ml of fluid were collected from each of eight full term horse placentas. All eight fluids, individually tested after filtration, had hemagglutination-inhibition (HA-I) titers of approximately 1:640 to H-1 virus.

The hemagglutination-inhibition tests, disc electrophoretic techniques, treatment with enzymes and sugars and the chemical techniques employed have been described in detail previously (3,4).

Purification of the Inhibitor. The purification scheme followed in the present work closely resembled the one previously described (3,4) for isolation of the human placental inhibitor. The ammonium sulfate precipitation step, however, was omitted since it had been determined that the amounts of protein obtained from the horse fluids by this procedure were minute.

Results. The purification procedure used produced a final product that represented less than 0.01% of the original protein, yet contained at least 57% of the original activity (Table I). The kaolin absorption step, as expected, was necessary for removal of nonspecific inhibitors. Preparative ultracentrifugation concentrated and separated the inhibitor from the bulk of lighter placental fluid proteins. Ammonium sulfate precipitation was omitted since less than 5% of the proteins in the centrifugation pellet could be separated by salting out (apparently gamma globulins are absent from horse placental fluid since no band was found in the gamma globulin area in repeated tests of such fluids with disc electrophoresis). The behavior of the inhibitor when examined by gel filtration in a Sephadex G-200 column and by sucrose den-

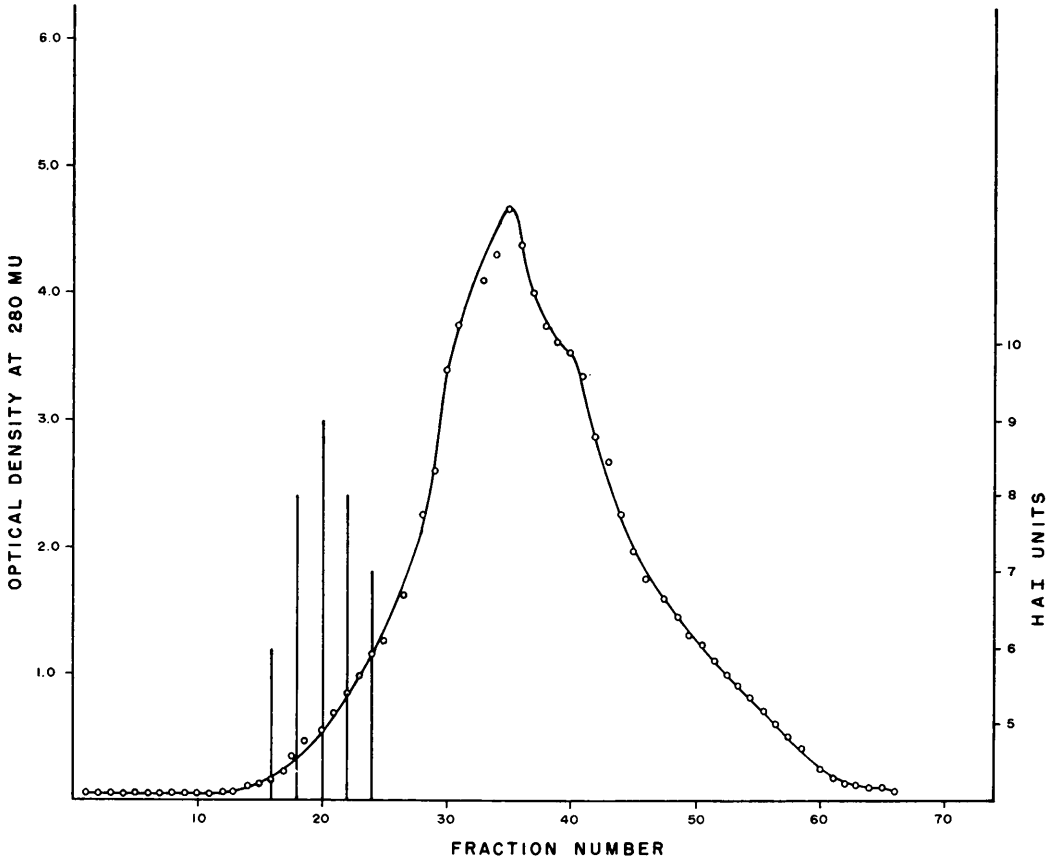

FIG. 1. Elution diagram of Sephadex G-200 column fractions.

sity centrifugation (the last steps in the purification scheme), suggests that this substance might be a 19S macroglobulin. Inhibitory activity (Fig. 1) appeared in the first fractions eluted from the Sephadex G-200 column, i.e., the macroglobulin area, while sucrose gradient centrifugation (Fig. 2) showed that the horse inhibitor sediments in the region corresponding to the site of the 19S macroglobulins of serum.

Fractions obtained at each step in the purification procedure were examined by disc electrophoresis. The fraction obtained after kaolin absorption and also the active sucrose gradient fractions were stained with amido black, as illustrated in Fig. 3. Staining with oil red O gave negative results. On the other hand, staining with Schiff reagent gave a positive result.

The inhibitor is remarkably stable to heat, for no loss of HA-I activity occurred when either the kaolin extracted fluid or Sephadex active fractions were heated at 100°C for 1 hour (Table II). Treatment with pepsin, chymotrypsin, or trypsin for the same length of time, also had no effect on the HA-1 titer. However, treatment for 1 hour with papain destroyed the inhibitor activity. Neuraminidase also destroyed 90% of the activity while sodium metaperiodate completely inactivated the inhibitor. The *N*-acetylglucosamine rhamnose, mannose, and galactose interfered with the reaction of inhibitor and virus as shown by loss in HA-I titer. Treatment with mercaptoethanol was ineffective.

It is noteworthy that the final purified fractions were active in a concentration of only 0.006 μg/ml.

Discussion. It has been shown that horse serum is capable of inhibiting the hemagglutinating action as well as the infectivity of the Asian influenza viruses (8,9). Attempts have

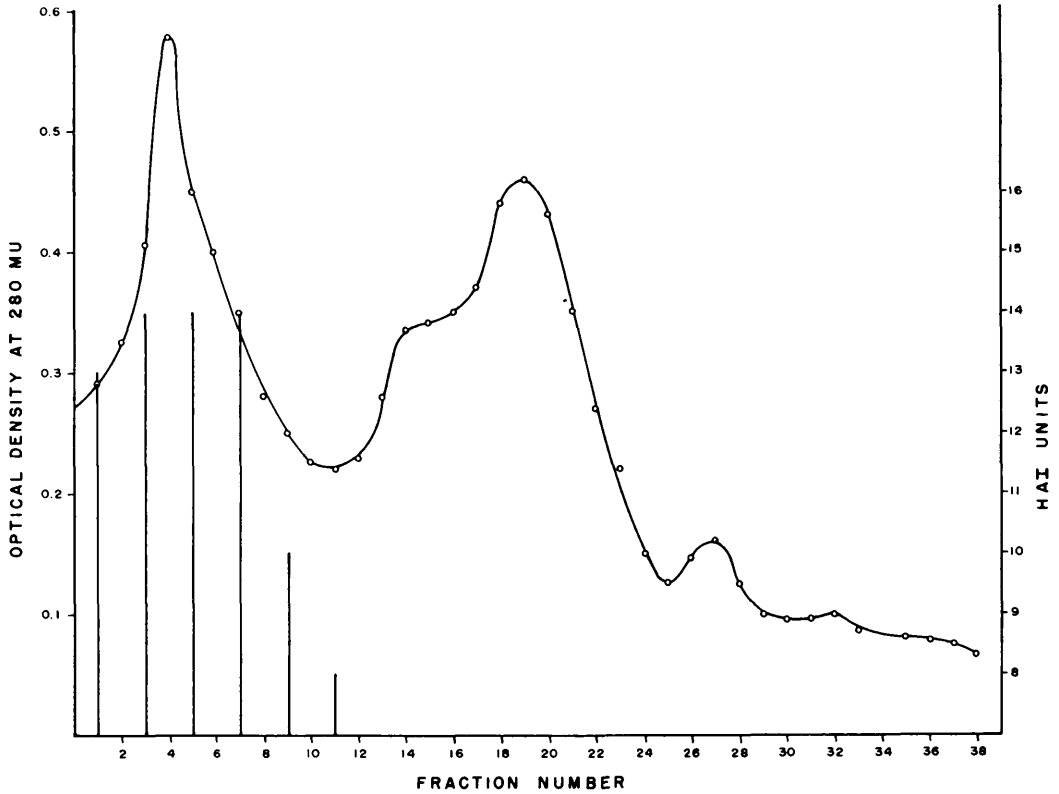

FIG. 2. Sucrose density gradient diagram of active Sephadex G-200 column fractions.

been made to purify the active material by a number of investigators who have reported on final preparations which vary in their electrophoretic mobility and in their sedimentation properties (8). The serum inhibitor which, according to Biddle et *al.* (8), occurs naturally as a fragile 18S component, is apparently a glycoprotein containing sialic acid (9). It is readily activated by heat. Certain normal horse sera also inhibit the multiplication of poliovirus in tissue cultures (10). The active inhibitor is bound to the viral surface *in vitro* in the absence of tissue cells, and is a 19S macroglobulin. It is also a glycoprotein.

The horse placental inhibitor which, in contrast to the above, is absent from horse sera, also sediments as a macroglobulin during density gradient ultracentrifugation. When examined by disc electrophoresis, it behaves as a homogeneous product. Whether the single band obtained represents one pure protein or is contaminated with other serum macroglobulins, as in the human (4), has not been determined. Present studies indicate that horse and human placental fluids differ in their protein composition. Horse placental fluids, for example, in contrast to human placental fluids, do not contain gamma globulins (Fig. 3). By salting out the human fluids with ammonium sulfate a considerable amount of α_2M macroglobulin contamination could be removed. This step was omitted in the present purification since it was found that the only proteins salted out were smaller molecules which could be removed by column chromatography. Another macroglobulin contaminating the human placental inhibitor fractions after kaolin absorption was IgM globulin. Apparently, there are no substances in the horse placental fluids comparable to the α_2M and IgM macroglobulins of the human placental fluids. The present findings indicate indirectly that the disc electrophoret-

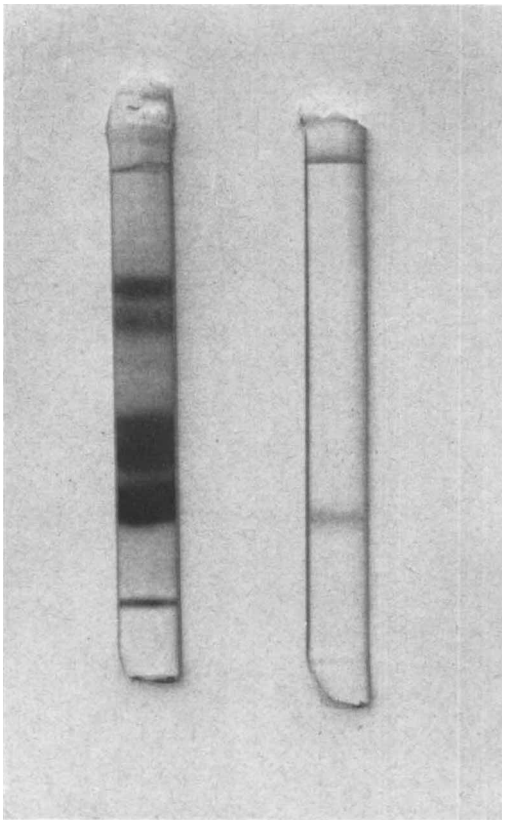

FIG. 3. Disc electrophoresis protein patterns of the horse placental kaolin-absorbed fluid and the active sucrose gradient fractions.

ic homogeneous active fraction of the horse placental fluids may represent a single protein, namely, the pure inhibitor.

Loss of activity for the horse placental inhibitor after treatment with papain, neuraminidase, or sodium metaperiodate indicates that the inhibitor is a glycoprotein as are the horse serum inhibitors of hemagglutination by other viruses (8,9,10). Lack of activity after the neuraminidase or periodate treatment indicates not only that the placental fluid inhibitor is a glycoprotein, but also presents the possibility that sugar groups in the molecule are involved in the relationship of the inhibitor with H-1 virus. This possibility is supported by interference of rhamnose, galactose, mannose, and *N*-acetylglucosamine with the reaction of inhibitor, virus, and red cells. The fact that mercaptoethanol did not affect the inhibitory activity suggests that the purified material is not an IgM globulin.

The glycoprotein inhibitor obtained from the horse also differs from the human inhibitor in its reaction with proteolytic enzymes. While one hour treatment with trypsin and chymotrypsin does not alter the HA-I activity of the horse glycoprotein, it completely destroys that of the human. Therefore, though both the human and horse inhibitors are glycoproteins and sediment as macroglobulins, the chemical structures of the two substances must be different.

Summary. The active material found in horse placental fluids which inhibits the hemagglutination of H-1 and HB viruses has been purified, and a homogeneous product obtained as shown by disc electrophoresis. The inhibitor sediments in a sucrose gradient as a macroglobulin that can be rendered inactive by treatment with papain, neuraminidase, or sodium metaperiodate. Though a similar inhibitor is found in human placental fluids, the two substances are not identical. The purified material from the horse placental fluids shows a remarkable capacity for

TABLE II. Sensitivity of the Hemagglutination Inhibitory Activity to Various Treatments.[a]

Treatment	HA-I titer Before	After
A. Chemical		
Trypsin	640[b]	640
Chymotrypsin	640	640
Papain	640	0
Pepsin	640	640
Neuraminidase	320	40
$NaIO_4$	160	0
2-Mercaptoethanol	640	640
Mannose	640	0
N-Acetylglucosamine	640	320
Rhamnose	640	320
Galactose	640	160
Glucosamine	640	640
B. Heat		
56, 75, 85, or 100°C for 1 hour	640	640

[a] A pool of active Sephadex column fractions was used for the above treatments.

[b] Guinea pig cells were employed for these procedures. Results are expressed as the highest dilution that completely inhibited hemagglutination.

inhibiting hemagglutination by H-1 virus since it is active in concentrations as low as 0.006 μg/ml.

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A Study of Estrogen-Sensitive Hypothalamic Centers Using a Technique for Rapid Application and Removal of Estradiol* (33000)

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Harris *et al.* (1) reported eliciting estrous behavior in spayed female cats by placing stilbesterol implants in the hypothalamus. Various workers since have investigated the effects of sex steroids on estrogen sensitive centers in the cat (2) rat (3,4) and rabbit (5). From the work of Lisk (3,4) it now seems quite evident that in the rat there are two estrogen-sensitive centers in the hypothalamus. One, located anteriorly and just dorsal to the optic chiasm, is concerned with initiation of sex behavior. The second center, located in the median eminence region of the posterior hypothalamus is apparently concerned with the release of gonadotropins from the pituitary. The female rabbit appears to differ from the above named species in that estrous behavior has been reported when estrogen was implanted into the ventromedial nucleus of the hypothalamus (5).

Materials and Methods. Previous studies have been carried out in groups of animals with permanently implanted tubes. We have devised a simple method which makes it possible to remove estrogen implants. A 6-mm length of 22-gauge stainless steel tubing is cut and polished. This short piece of tubing, which we call a "guide barrel" is stereotaxically placed in the calvarium and firmly affixed by means of dental cement. A piece of 27-gauge tubing attached to the stereotaxic chuck serves as a carrier for the "guide barrel." After the placement holes have been drilled, the "guide barrel" is slipped down the 27-gauge carrier tube into the brain, leaving a 3-mm projection above the surface of the calvarium, the 3-mm line having previously been measured and marked. Dental cement is then applied, and once hardened, holds the "guide barrel" in a rigidly fixed position. Following placement of the "guide barrel," a short piece of 27-gauge tubing, bent at one end for easy removal with forceps, is placed in the 22-gauge tubing and serves as a plug (Fig. 1).

The estrogen-tipped tubes were prepared for implantation in the following way. Estradiol was brought to the melting point and by means of polyethylene tubing with a hypodermic syringe attached to one end of the steel tubing, a negative pressure was created to

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