be used successfully for both induction of interferon and assay of interferon action clearly shows that certain strains of this virus are both stimulatory and responsive in the interferon system.

Existence of an interferon produced by cells infected with HSV suggests that interferon may play an important role in human infections of HSV. Since herpes antibody titers do not vary significantly in humans prior to or following the recurrence of lesions (4,11), it must be assumed that some other factor is responsible for the host-virus equilibrium. Results obtained in our laboratory (8) have demonstrated that antibodies play a secondary role in the course of herpes infection *in vitro,* and that interferon exerts the major effect in determining the outcome of such infections. Thus, it is quite conceivable that interferon may play a predominant role in the classic latency of herpes in humans.

Summary. The production of an interferon-like substance by chick embryos infected with herpes simplex virus is described. The physical, chemical, and biological properties of herpes interferon are demonstrated to be similar to those of previously reported interferons. Speculation is made on the possible role of interferon in the classic example of latency in humans—infection by herpes simplex virus.

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Received June 15, 1964. P.S.E.B.M., 1964, v117.

Effects of Retrograde Injections of Vasopressin into the Upper Urinary Tract of Hydrated Rats. (29704)

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That arginine-vasotocin can act when delivered to the tubular cells from the blood side has been shown by infusing the hormone into the renal portal circulation of the chicken(3). It has also been shown that retrograde flow into the distal part of the nephron can occur when a slight hydrostatic pressure is applied(1,2,4). In the present study arginine-vasopressin was injected through the ureter in water-loaded rats to try to determine whether the hormone can act when present only on the luminal side of the distal tubules and collecting ducts.

Materials and methods. Trained male white rats weighing about 400 g were fasted overnight, then 4 ml/100 g of 12% (v/v) ethanol in water was given by stomach tube. Forty-five minutes later 5-10 mg/100 g of Inactin® (Promonta, Hamburg), a thiobarbituric acid derivative, was injected intraperitoneally. The trachea was cannulated, then 5 ml/100 g of 2% (v/v) ethanol in water was given orally. A lower median laparotomy was performed. No. 10 PE catheters were passed into both ureters approximately to a point 5 mm from the pelvis; the ureters were ligated, and the laparotomy was closed. The right catheter was connected to a mercury manometer through a side tube. A hydration of approximately 8% of body weight was maintained throughout using the 2% ethanol solution. When a good water diuresis (osmolality about 100 mOsm/kg) had been established after the operation, retrograde in-

jections of the following solutions were given:

1. A control solution of sodium chloride having an osmolality of 100 mOsm/kg,

2. Synthetic arginine-vasopressin (Ferring lot no. 20 411) dissolved in the control solution to a concentration of 20 mU/ml, and,

3. India ink (Pelikan Spezialtusche C 11/1431 a, Günther Wagner) diluted with the control solution 1:6 (v/v).

Approximately 0.5 ml was injected in order to reach and maintain a pressure of 20-50 mm Hg. The antidiuretic action of the hormone dilution was controlled by i.v. injection in the same rat.

In some experiments the injection of the control solution resulted in a pronounced bilateral antidiuresis. Such experiments were discarded, and the rats were given more ethanol and Inactin, until the control injection could be made without causing antidiuresis. When a number of injections of vasopressin had been given, the India ink solution was injected with exactly the same pressure and duration as the injections of the control and hormone solutions. Before the injection was completed, the abdomen was quickly opened, the renal stalk clamped, the kidney removed with the clamp on and placed in iso-pentane cooled in dry CO_2. This procedure took no more than 10 seconds. The frozen kidney was transferred to the Pearse-Slee cryostat and cut in 8 μ sections which were allowed to dry in the cryostat (—20°C). The dry sections were fixed with formalin and cautiously stained with hematoxylin-eosin. The kidney was cut in the midline through the papilla with the cut surface at right angles to the long axis of the kidney, thus exposing the collecting ducts longitudinally. A number of sections were examined from each kidney, both before and after staining. The staining procedure was not observed to cause any displacement of the India ink.

The urine was collected in small tubes at 4-10 minute intervals. The tubes were tightly closed immediately after the collection. Osmolality was determined on 0.25 ml samples using an Advanced Instruments thermistor "osmometer" (model 31 LS) and NaCl solutions as standards. A standard deviation of 0.90 mOsm/kg was found with the method using rat urine. This gives a coefficient of variation of 1% of the osmolalities obtained by this procedure.

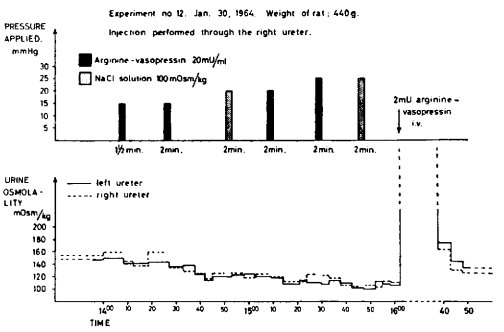
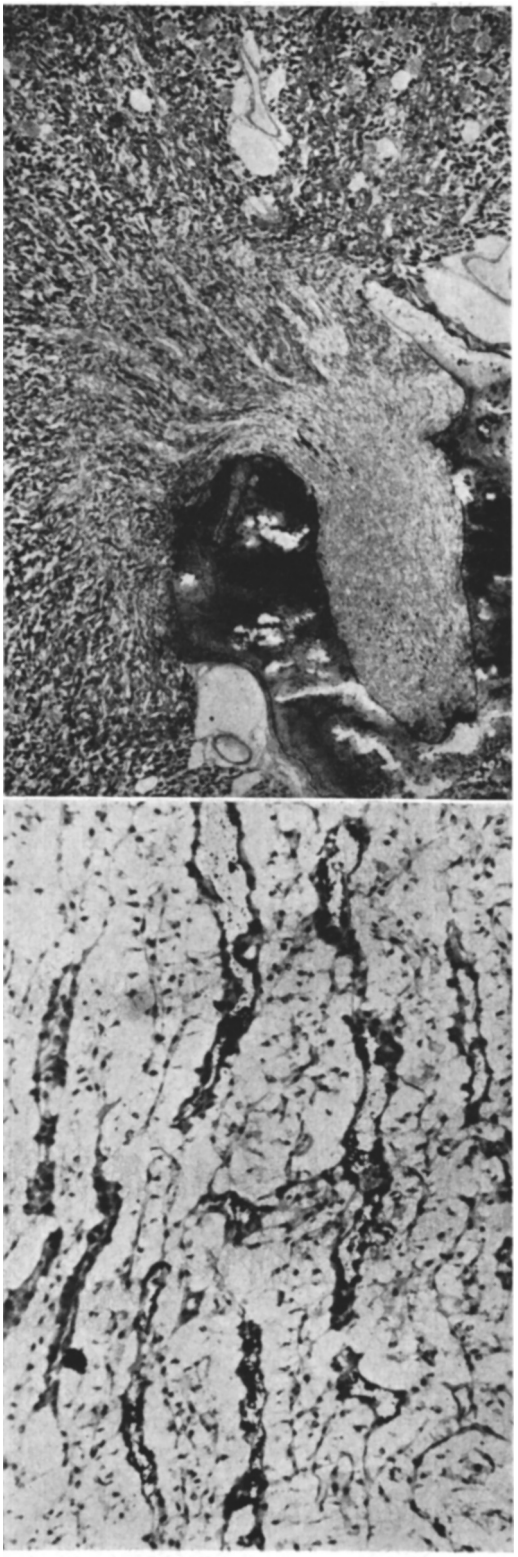


FIG. 1. Exp. 12. Urine osmolality during 4 retrograde injections of arginine-vasopressin, and 2 control (NaCl) injections. An i.v. injection is performed in order to control antidiuretic activity of hormone solution.

The controls and the hormone solutions were stained with methylene blue in order to distinguish between the solution injected and the new urine formed after the release of flow. The first urine fraction after the release of flow was collected from the moment when no blue color could be detected visually.

Results. In preliminary experiments it was found that the maximal ureteral pressure created by complete obstruction was less than 20 mm Hg. The stained solution was found to run up the ureter when the pressure surpassed 8-15 mm Hg. When pressures over 50 mm Hg were applied a rapid infusion took place. The kidneys from such experiments showed pronounced damage especially at the outer papillary-medullary junction. Consequently, pressures between 20 and 50 mm Hg were used. The duration was 1-3 minutes. A total of 12 injections in 4 rats (where the histological examination of the kidney showed India ink to be present in the papilla in the slices examined) were selected for analysis of osmolality. A typical experiment is shown in Fig. 1 (from Exp. 12). A typical histological picture is shown in Fig. 2 (from Exp. 11 and 12). Approximately 15% of the collecting ducts contained India ink in these experiments. In no case was any unilateral of contralateral antidiuresis observed.



In some experiments (5 injections in 3 rats) an enormous concentration (20 IU/ml) of lysin-vasopressin (Insipidin,® a commercial preparation from hog posterior pituitaries) was injected. This resulted in a slight bilateral antidiuresis without any preponderance on the side into which the injection had been made. This antidiuresis was probably due to absorption of the hormone from the ureter and pelvis, or penetration into the kidney lymphatics.

Histological examination showed the India ink always present in a few collecting ducts in the inner and sometimes in the outer papilla. The number of collecting ducts filled as judged visually from good slices showing the papilla could not be correlated with the state of diuresis, pressure applied, or duration of obstruction (a total of 10 kidneys were examined). In some kidneys the papilla contained very little India ink. Such experiments were excluded.

Discussion. No ipsilateral antidiuresis was demonstrated by this method of retrograde injection. Whether or not such a finding proves that the hormone acts from the urine side to increase the permeability depends on two main questions: 1. Has the hormone been present in an adequate concentration for an adequate period to be bound to the cells? 2. Have a sufficient number of collecting ducts been filled with solution containing vasopressin? 200 μU/100 g rat injected i.v. results in a maximal antidiuresis(5). Suppose this amount is distributed only in the plasma volume (5% of body weight) and excreted unchanged in the urine. Since the urine flow during water diuresis is more than 1% of the glomerular filtration rate the hormone is concentrated less than 100 times from plasma to urine, *i.e.* from 40 μU/ml to less than 4 mU/ml. As the concentration used here was 20 mU/ml (or slightly smaller due to dilution with the ureteral urine) and the time during which the collecting ducts were exposed to the hormone was much longer than the passage from ultrafiltration

FIG. 2. Histological picture showing India ink in collecting ducts. Hematoxylin and eosin. Top: Exp. 11 (× 13). Bottom, Exp. 12 (× 112).

to ureteral excretion, the first question may be answered in the affirmative.

The method of preparation insures that the India ink observed in the collecting ducts has actually been present there *in vivo*. This was further confirmed by examining a number of sections from the same kidney. But it is quite clear from the microscopic examination that only some collecting ducts were filled. Since a number of injections of hormone were given to all rats before the injection of India ink, it is assumed that the hormone-containing solution, at least during some of the injections, penetrated as far up into the papilla as did the India ink. For these reasons it would seem safe to conclude that the hormone does not have any marked effect from the luminal side of the collecting ducts in the papilla of the rat, since such an effect would have caused an increased osmolality of the urine.

Summary. Unilateral, retrograde injection of arginine-vasopressin into the ureter of the hydrated rat did not result in a detectable increase in urine osmolality. The degree of penetration into the tubule was controlled by injection of an India ink solution and later histologic examination. Although only some collecting ducts could be assumed to be filled with hormone-containing solution the finding suggests that the hormone has no marked effect from the luminal side of the collecting ducts in the papilla of the rat.

The author cordially thanks Dr. J. Kazimierczak, The University Institute of Pathology, Copenhagen, for preparing the microscopic sections.

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Received June 8, 1964. P.S.E.B.M., 1964, v117.

A Hemolytic Factor Associated with *Leptospira pomona* Cells.† (29705)

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A hemolysin associated with the culture supernatant fluids of leptospirae has been reported by several investigators(1,2,3,4). However, these workers reported very little hemolytic activity associated with the leptospiral cell. Valentine and Morter(5) reported considerable hemolytic activity associated with the cells of some isolates of *Leptospira pomona*. The partial purification and preliminary characterization of this hemolytic factor is the subject of this report.

† Paper submitted as Journal Series paper 2351, Purdue Univ. Agr. Exp. Sta., Lafayette, Ind., and investigation supported in part by U.S.P.H.S. grant AI04029 from I.A.I.D.

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Materials and methods. The sources of the 19 isolates of *L. pomona* used in this study are given in Table I. Evaluation of the hemolytic factor of the cells and culture supernatant fluids of all isolates were undertaken and further studies were made of cellular hemolytic factor of strain P-7, the hamster lethal Wickard strain(6).

The *L. pomona* isolates were cultivated in Chang's medium(7) for 10 days at 28°C in Roux bottles. Each bottle was accepted for hemolysin evaluation if found free of contamination by test with fluid thioglycollate medium, tryptose broth in Castaneda flasks and blood agar plates (5% bovine blood).

L. pomona cells were concentrated by cen-