14. Pedersen, K., *Arch. Biochem. Suppl.*, 1962, v1, 157.
15. Sober, H. A., Peterson, E. A., *Fed. Proc.*, 1958, v17, 1116.
16. Tan, M., Epstein, W. V., *Science*, 1963, v139, 53.
17. Fleischman, J. B., Porter, R. R., Press, E. M., *Biochem. J.*, 1963, v88, 220.

Received June 2, 1964. P.S.E.B.M., 1964, v117.

Response of the Superficial Lymphatics of the Arm of Intact Man to Synthetic Vasopressin.* (29519)

G. E. BURCH AND N. P. DEPASQUALE
Department of Medicine, Tulane University School of Medicine, Charity Hospital of Louisiana, and Veterans Administration Hospital, New Orleans

Within the past few years advances in the field of polypeptide chemistry have permitted identification and synthesis of a number of very potent vasoactive polypeptides(1). During studies with 2 synthetic vasopressins phenylalanine^2-lysine^8-vasopressin (PLV-2)† and lysyl^8-vasopressin (Lys-Vp)†, an unusual response to the intradermal injection of these substances was noted.

Material and methods. PLV-2. PLV-2 was injected intradermally on the medial aspect of the volar surface of the forearm approximately 15 cm proximal to the wrist of 10 human subjects varying from 24 to 66 years of age. In addition, PLV-2 mixed with patent blue V dye was injected intradermally at a similar site on the volar aspect of the forearm of another 15 subjects ranging from 24 to 53 years of age. Control injections of dye diluted with 0.85% sodium chloride solution were made in the opposite forearm. The technic for studying the superficial lymphatics with vital dyes, such as patent blue V, was first described by McMaster(2) and has been used for many years in this laboratory(3).

In the experiments in which PLV-2 was injected without dye, a solution containing 5,000 milliunits (mU) of PLV-2 per cc was used. The volume of fluid injected varied between 0.01 cc (50 mU of PLV-2) and 0.1 cc (500 mU of PLV-2). In the majority of experiments 0.01 cc of fluid was injected. In the experiments in which PLV-2 was mixed with patent blue V dye, 0.5 cc of an 11% solution of patent blue V dye was added to 0.5 cc (2,500 mU) of PLV-2 to make a solution containing 2,500 mU of PLV-2 per cc in 5.5% patent blue dye. From 0.01 cc (25 mU of PLV-2) to 0.1 cc (250 mU of PLV-2) of this mixture was injected intradermally. Control injections of dye were made with a 5.5% stock solution of patent blue V; the volume injected being equal to the volume of the test fluid injected in the contralateral forearm.

All injections were made with a specially constructed microsyringe which made it possible to introduce small volumes of fluid with accuracy(4). After an intradermal wheal was raised records of the responses to the polypeptide and the dye were made by placing a clear strip of exposed X-ray film over the arm and tracing directly onto the film the various responses observed at 5 minute intervals. Color and black and white photographs of the forearm were also obtained at various intervals to record the rate of the responses to the intradermal injections.

All subjects were studied in a climatic room maintained at 22° to 24° Fahrenheit and 46% relative humidity. The subjects' arms were supported passively at heart level throughout the observations.

Lys-Vp. Lysyl^8-vasopressin (Lys-Vp) was injected intradermally on the forearm as de-

* Supported by grants from U. S. Public Health Service, Rudolph Matas Memorial Fund for the Kate Prewitt Hess Laboratory and Rowell A. Billups Fund for Research in Heart Disease.

† Supplied by Dr. Rudolph Bircher, Sandoz Pharmaceuticals.

scribed above for PLV-2 in 5 subjects ranging from 36 to 60 years of age. Lysyl[8]-vasopressin mixed with patent blue V dye was injected in another 5 subjects ranging from 42 to 63 years of age. The concentration of Lys-Vp used in these experiments was adjusted so that 1.0 cc of solution contained 5,000 mU of Lys-Vp. Injections without dye consisted of 0.1 cc of fluid containing 500 mU of Lys-Vp, whereas injections with dye consisted of 0.1 cc of fluid containing 250 mU of Lys-Vp.

The experimental conditions including the injection technic were exactly the same as described above for the experiments with PLV-2.

Results. I. Intradermal injection of PLV-2. PLV-2 alone was injected intradermally in 10 subjects. Although there were some individual variations in response to the intradermal injections of PLV-2, the pattern of response was uniform in the majority of subjects. A typical response to injection of 0.01 cc (50 mU) of PLV-2 was as follows:

One minute after injection. Almost immediately following the formation of an intradermal wheal with PLV-2, an area of blanching developed around the margin of the wheal, which extended 0.5 to 1.0 cm beyond the edge of the wheal. The area of blanching, in turn, was surrounded by an area of minimal to moderate, but never intense, erythema. The area of erythema extended 1.0 to 2.0 cm beyond the blanched area and was well circumscribed but had an irregular border.

Five minutes after injection. The size of the intradermal wheal increased with some elevation of the dermal surface, due to edema, to include a portion of the marginal blanched area. As the wheal "lifted," it also blanched, especially at its center. There was no change in the area of erythema (Fig. 1).

Five to 15 minutes after injection. One to 4 pale streamers began to extend from the blanched area penetrating the area of erythema (Fig. 1). The wheal continued to increase slightly. The area of erythema remained unchanged except for the pale streamers which extended through it from the area of blanching.

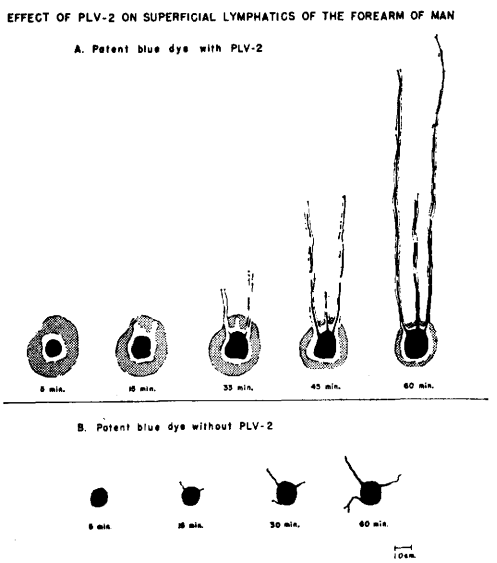

FIG. 1. Tracings showing a typical response to intradermal injection of 250 mU of PLV-2 mixed with patent blue V dye (A) and to patent blue V dye without PLV-2 (B). Black area represents location of the dye, white area represents region of blanching and stippled area represents region of erythema. Within 15 min of injection pale streamers extend out from the blanched area through the area of erythema. Soon after the development of these pale streamers, dye begins to appear in the center of the pale streamers and extends up the arm to reach the axilla in one hour. When patent blue V dye alone was injected (B) pale streamers did not develop and blue streamers extended only 0.5 to 4.0 cm from injection site in one hour.

Fifteen to 30 minutes after injection. The pale streamers continued to extend outwards from the marginal blanched area and by 15 to 30 minutes had extended well beyond the area of erythema (Fig. 1). The best developed streamers extended superiorly up the arm, whereas the shorter and less developed streamers extended inferiorly or laterally from the site of injection.

Thirty to 45 minutes after injection. By this time, one, two, or more elongated pale streamers extended 5 to 10 cm from the site of injection. The area of erythema began to fade slightly, whereas the blanched or pale area remained essentially unchanged.

Forty-five to 60 minutes after injection. The pale streamers had reached their maximal length, often extending into the axilla

or reaching a high point on the antero-lateral or antero-medial aspect of the arm. The area of erythema had faded and was barely perceptible, whereas the initial blanched area remained unchanged. The wheal had not changed in diameter but its volume had diminished as the edema subsided and the surface of the wheal became flat.

Sixty minutes to 3 hours. During the next 3 hours the pale streamers slowly disappeared. However, in several subjects streamers were still faintly perceptible at the end of the 3-hour observation period. In all subjects the intradermal wheal completely disappeared by the end of 3 hours.

Comment. The most striking feature of the response to the injection of PLV-2 was the development of from 1 to 4 pale streamers in the skin which extended up the arm away from the injection site. The length of the pale dermal streamers varied from 8.0 cm to 21.0 cm (average 16.2 cm). Although we had never observed such a response in previous studies on the lymphatics of man it was considered that the lymphatics were in some way involved in streamer formation. The pale streamers spread too rapidly and were too sharply outlined to have been caused by dissection of PLV-2 through the tissues from the site of injection. If the reaction had been due to collection of PLV-2 by the veins it should have been self-limiting since venoconstriction (which would have to be postulated to explain the paleness of the streamers) would have prevented further propagation of the polypeptide. In addition, as far as could be determined the streamers did not follow the veins. It was therefore postulated that PLV-2 entered the lymphatics and flowed through these channels. Furthermore, the pale streamers in the skin suggested a local constriction of the perilymphatic blood vessels. In order to identify more definitely the nature of the pale streamers, the next phase of these experiments were conducted with PLV-2 mixed with patent blue V dye.

II. Intradermal injection of PLV-2 with patent blue V dye. These experiments were conducted precisely as those described above except that the PLV-2 was mixed with patent blue V dye just prior to its injection. Five to 10 minutes after the pale streamers developed, blue-tinged streamers began to extend from the wheal, coursing in the center of the pale streamers (Fig. 1, 2). The pale streamers always extended well ahead of the blue streamers until the pale streamers reached their final lengths. Then the blue streamers "caught up" with the pale ones to extend the full length of the pale streamers. The pale streamers measured 2.0 to 3.0 mm in diameter. Because of the pallor of the larger streamers the centrally located blue streamers could easily be seen. The blue streamers varied between 6.0 and 30.0 cm in length (average 16.8 cm).

Comment. From previous studies of McMaster(2) and from this laboratory(3) it is known that patent blue V dye is collected by the lymphatic vessels. The fact that the pale streamers always contained centrally located blue streamers as well as the fact that blue streamers never appeared outside of a pale streamer indicate that the "core" of the pale streamer was a lymphatic vessel. The "lag" in appearance of the blue streamer was probably related to the time necessary for sufficient dye to accumulate within the lymphatics to render them grossly visible. The rapidity with which the blue streamers developed as well as their relatively great length suggested that PLV-2 had increased the rate of lymph flow whereas the pallor in the skin must have been due to constriction of perilymphatic blood vessels.

III. Intradermal injections of PLV-2 without dye followed by injection of patent blue V dye. To test the hypothesis that the rate of lymph flow was increased by PLV-2, pale streamers were produced by injection of 500 mU of PLV-2 without dye. One hour after the pale streamers had developed patent blue V dye was injected into the same site as had the PLV-2. Within seconds of the injection of the patent blue V dye the dye was noted to enter the pale streamers and completely fill the entire length of the pale streamers within 2 or 3 minutes.

Comment. The extremely rapid rate at which patent blue dye extended along the

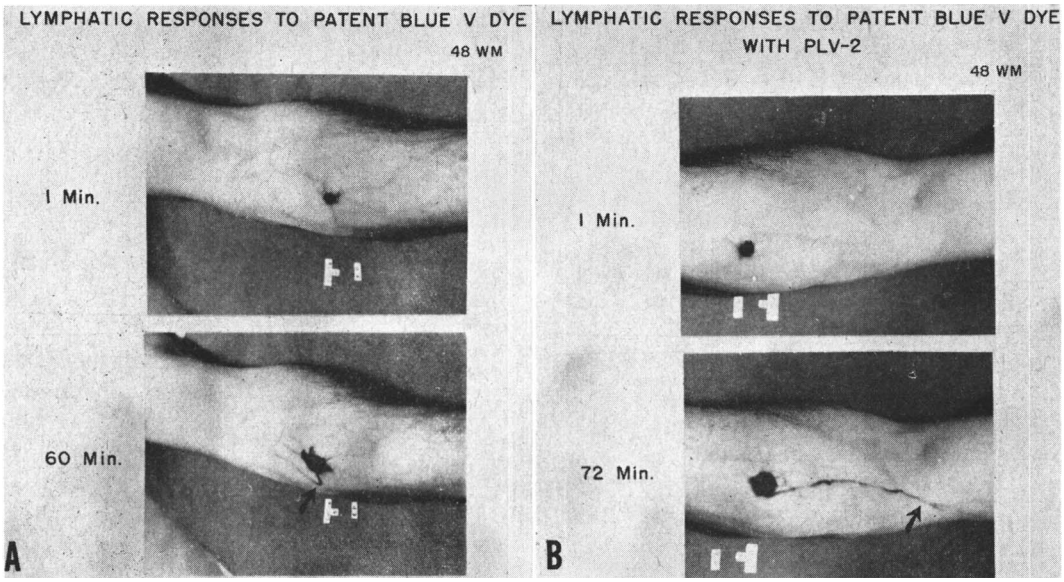

FIG. 2. (A) Photographs taken with a Wratten series VII filter of a typical response to intradermal injection of patent blue V dye without PLV-2. Because of the Wratten filter the veins appear prominent. A streamer containing dye is indicated by arrow. The greatest length of any streamer at the end of one hour was 3.5 cm. (B) Photograph showing a typical response to intradermal injection of patent blue V dye with 250 mU of PLV-2. A streamer containing dye is indicated by the arrow. Although the streamer measures only 13 cm in the photograph, it actually extended 22 cm to the axilla. The pale component of the streamer cannot be seen in photograph because of the filter.

entire length of the pale streamers suggested that the rate of lymph flow was increased. In 2 subjects the patent blue dye could be identified in the axilla before the injection of dye was even completed.

IV. Intradermal injections of normal saline and of normal saline mixed with patent blue V dye. A wheal was raised on the forearm by intradermal injection of 0.01 cc of 0.85% sodium chloride solution in the same manner as described for the above experiments. In some subjects the wheal became surrounded by an area of blanching immediately at the base of the wheal. However, in all of the subjects studied the dermal responses subsided within 10 minutes after injection of saline.

The injections of saline mixed with patent blue V dye were associated with the formation of small streamers of dye, which after one hour extended 0.5 to 12.0 cm (average 4.7 cm) beyond the site of injection (Fig. 1). The response was similar to that observed many times in this laboratory after intradermal injection of patent blue V dye and completely unlike that associated with the injection of PLV-2 mixed with patent blue V dye (Fig. 1).

Comment. The rate of streamer formation and lengthening when patent blue V dye was injected without PLV-2 averaged 4.7 cm at the end of one hour, whereas that with patent blue dye mixed with PLV-2 averaged 16.8 cm at the end of one hour. Thus, the PLV-2 did increase the rate of streamer formation and presumably the rate of lymph flow. The possibility that the edema which developed at site of injection of PLV-2 was responsible for opening the lymphatic channels was considered. However, this was unlikely on the basis of previous studies with the polypeptide bradykinin and with histamine in which intradermal injections of these substances were associated with marked local edema formation with no acceleration in the rate of streamer formation and lengthening (unpublished observations of the authors).

V. Intradermal injection of Lysyl^{8}-Vasopressin with and without patent blue V Dye. The dermal vascular responses to intradermal

injection of 500 mU of lysine vasopressin were identical to those described above for PLV-2.

Intradermal injection of Lys-Vp mixed with patent blue V dye were also identical to those described for PLV-2 mixed with patent blue dye. The length of the blue streamers at the end of one hour varied between 6.5 cm and 30.0 cm (average 17.7 cm).

Comment. The dermal vascular responses observed for Lys-Vp were identical to those described for PLV-2. No differences in rates of development of pale and blue streamers or in duration of the response between injections of PLV-2 and Lys-Vp were observed.

Discussion. These experiments indicate that the rate at which streamers of patent blue V dye extend away from an intradermal injection site on the volar aspect of the forearm is accelerated by mixing either PLV-2 or Lys-Vp with the patent blue V dye. On the other hand, intradermal injections of other vasoactive polypeptides including bradykinin, oxytocin and angiotensin II did not accelerate the rate of blue streamer formation (unpublished observations). Since patent blue V dye traces the lymphatics, it is reasonable to assume that PLV-2 and Lys-Vp increased the rate of lymph flow. The mechanism by which the rate of lymph flow was increased is unknown. The relatively slight increase in volume of the bleb at the site of injection could not have been responsible for the increased rate of lymph flow since substances such as bradykinin and histamine which produce much more edema at the site of injection than PLV-2 and Lys-Vp do not accelerate the rate of blue streamer formation. Possible mechanisms by which PLV-2 and Lys-Vp may accelerate the rate of lymph flow include dilation of the lymph channels, increase in peristalic activity of the smooth muscle of the lymphatics and closure of lymphatico-venous communications.

Summary. Preliminary studies indicate that PLV-2 and Lys-Vp, 2 synthetic vasopressins, increase the rate of lymph flow in the superficial lymphatics of the forearm. The mechanism by which these polypeptides influence the rate of lymph flow is unknown.

1. Lewis, G. P., *Physiol. Rev.*, 1960, v40, 647.
2. McMaster, P. D., *Bull. N. Y. Acad. Med.*, 1942, v18, 751.
3. Burch, G. E., *Anat. Rec.*, 1939, v73, 443.
4. ———, PROC. SOC. EXP. BIOL. AND MED., 1939, v40, 676.

Received June 2, 1964. P.S.E.B.M., 1964, v117.

A Plaque Reduction Neutralization Test for Human Cytomegalovirus.* (29520)

GORDON PLUMMER AND MATILDA BENYESH-MELNICK
(Introduced by Joseph L. Melnick)
Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

Research on cytomegalovirus infections has been handicapped by the lack of typing antisera for the virus as well as by the tedious nature of the presently available methods for carrying out neutralization tests. For routine work with the large number of cytomegalovirus strains that have been isolated in this and other laboratories, neutralization tests are not practical when based on the cytopathic endpoint(1), the immunofluorescent focus assay(2), or the intranuclear inclusion body assay(3) methods.

A plaque assay is described here which has proven much more convenient. It has been applied successfully to the neutralization test employing sera obtained from human adults as well as immune antisera made in monkeys.

* This investigation was supported by research grants AI 05382 from Nat. Inst. of Allergy and Infect. Dis., and CA-04600 from Nat. Cancer Inst., and training grant 5 T1-AI 74 from Nat. Inst. of Allergy and Infect. Dis., N.I.H., U.S.P.H.S.