In the fibrinolytic activities examined the activator activity was found to exhibit a clear-cut increase. Iatridis *et al.*[5] expressed the belief that in exercise it is mainly the activity of lysokinase, that is, of a factor playing an integral part in the generation of the activator activity, that is increased.

This work was supported by the Sigrid Jusélius Foundation and the Finnish Medical Research Council.

[1] Cannon, W. C., and Gray, H., *Amer. J. Physiol.*, **34**, 232 (1914).
[2] Kesseler, K., and Wachholder, K., *Klin. Wschr.*, **35**, 1088 (1957).
[3] Wachholder, K., Parchwitz, E., Egli, H., and Kesseler, K., *Acta haemat.*, **18**, 59 (1957).
[4] Rizza, C. R., *J. Physiol.* (London), **156**, 128 (1961).
[5] Iatridis, S. G., Ferguson, J. H., Rierson, H. A., and Robinson, A. J., *Fed. Proc.*, **21**, 58 (1962).
[6] Sarajas, H. S. S., Konttinen, A., and Frick, M. H., *Nature*, **192**, 721 (1961).
[7] Biggs, R., Macfarlane, R. G., and Pilling, J., *Lancet*, i, 402 (1947).
[8] Ikkala, E., *Scand. J. clin. Lab. Invest.*, Suppl. 46 (1960).
[9] Björkman, S. E., *Acta haemat.*, **22**, 377 (1959).
[10] Nilsson, I., Blombäck, M., and von Franken, I., *Acta med. Scand.*, **159**, 35 (1957).
[11] Waaler, B. A., *Scand. J. clin. Lab. Invest.*, Suppl. 37 (1959).
[12] Astrup, T., Piper, J., and Rasmussen, J., *Scand. J. clin. Lab. Invest.*, **12**, 336 (1960).
[13] Amery, A., Vermylen, J., Maes, H., and Verstraete, M., *Thromb. Diath. haem.*, **7**, 70 (1962).
[14] Ogston, D., McDonald, G. A., and Fullerton, H. W., *Lancet*, ii, 521 (1962).
[15] Morehouse, L. E., and Miller, A. T., *Physiology of Exercise* (C. V. Mosby Co., St. Louis, 1959).

FUNCTIONAL VASCULAR FIELDS IN THE PITUITARY STALK OF THE MOUSE

By Dr. W. CURTIS WORTHINGTON, jun.
Medical College of South Carolina, Charleston

THE hypophyseal portal system of blood vessels has been amply demonstrated to be the morphological basis for the functional link between the hypothalamus and the anterior pituitary. The basic essentials of this system are as follows: (1) a set of primary capillaries (primary capillary 'plexus') in the neurohypophysis, particularly the median eminence and neural portion of the stalk; (2) those nerve fibres of the hypothalamico-hypophyseal tracts which have terminations near the primary capillaries; (3) a series of portal trunks connecting the primary 'plexus' with (4) the 'sinusoids' or secondary capillary 'plexus' in the anterior pituitary. There is an abundance of convincing evidence that neurohumoral substances from hypothalamic nerve endings pass by way of this system into the humoral environment of the glandular cells of the anterior lobe. (For recent reviews of the literature on neuroendocrine control of the anterior pituitary see Harris[1] and the Neuroendocrine Symposium, Miami, 1961 [2], and on the vascular anatomy and microcirculation, Worthington[3].)

Although much has been written concerning the morphology and anatomical distribution of these vessels, comparatively little is known of their physiology[4–7], and nothing is known concerning the possibility of functional specialization of separate vessels or groups of vessels within the system. If one considers particularly the primary capillaries, no evidence has been presented which goes beyond the concept of a functionally uniform network acting as a drainage unit for substances released from nerve endings. Implicit in most discussions of the role of the primary capillaries in the system is the conclusion that this capillary bed is functionally uniform throughout and the vessels freely anastomotic. There is little evidence indicating that there might be functional localization within this capillary bed.

It seems beyond question that the hypophyseal portal system taken as a whole is functionally unique and highly specialized. It also seems possible that specialization occurs within its major sub-divisions. The anatomical evidence concerning the primary capillary system is quite suggestive. The mouse shows an arrangement of groups of capillaries difficult to discern in injected or sectioned material but clear enough if the living stalk and median eminence are examined carefully, which suggests some degree of specialization. In man the vascular tufts and 'glomerular' formations which have been found in injected specimens suggest areas of specialized function[8–11]. Since there is now evidence that more than one substance is released into the primary capillary system for delivery to the sinusoids of the anterior lobe[12–14], and since there is no evidence to indicate whether or not parts of the median eminence and stalk are carrying out special functions, it seemed desirable to determine whether or not there are in the primary capillary area delimited vascular fields which might be functionally separable.

An investigation of this question was carried out by applying a phenomenon which was observed many years ago but has received little attention in recent times. In dead animals the neurohypophysis is intensely stained when injected with methylene blue in dilute solution. This was first described by Dandy[15] in 1913 and was discussed briefly by Bucy[16] in 1930, but seems to have been neglected since. The hypophyseal stalk can also be stained intensely in the living mouse.

In excess of 40 mice were used in this series of studies. The ventral surface of the pituitary stalk and median eminence and portions of the hypothalamus and anterior lobe were prepared under intraperitoneal sodium pentobarbital anæsthesia for direct microscopic observation by a procedure previously reported[4,5]. In brief, this consists of the careful exposure of the structures already described using a ventral approach through the neck. The pharynx, trachea and œsophagus are moved aside and a dental drill is used to make a window in the basisphenoid bone.

The pituitary stalks which have been studied in this series were examined either without further procedures or after the insertion of a small cannula (fashioned from a 30-gauge needle and a piece of *P.E.* 10 polyethylene tubing) into the femoral vein for the administration of a 1 per cent solution of methylene blue.

Observations were made with a Zeiss dissecting microscope giving magnifications of up to 40 times or a Bausch and Lomb binocular compound microscope equipped with 10× or 15× eyepieces and a 10× objective. Effective magnifications of 150 times are attainable with the latter, and resolution is much better than with the dissecting microscope. The Zeiss microscope has a built-in illuminator. A special lamp fitted with two infra-red absorbing filters was used for incident illumination with the compound microscope. In some of the early observations a Leitz Greenough binocular dissecting microscope was used rather than the Zeiss.

Of the more than 40 animals used for this work, 20 were subjected to the interruption of one or more arterial vessels to the median eminence or stalk. This procedure was carried out in the following manner: using number 000 insect pins as dissecting instruments, a very small hole was placed in the transparent dura mater in the vicinity of the blood vessel which had been chosen for

interruption. A second insect pin with a small hook at the tip (made by tapping the pin's point gently on a hard surface) was used to cut the vessel by placing the hook around it and lifting. On occasion sufficient bleeding was caused by this procedure to require that the animal be discarded, but this was unusual. Usually there was sufficient contraction and retraction of the cut tip that bleeding was either absent or minimal. The animals were prepared as outlined either with or without the interruption of small blood vessels and were then injected with from 0·01 to 0·02 c.c./g of body-weight of 1 per cent solution of polychrome methylene blue. Occasionally toluidine blue was used, but it seemed to offer no particular advantage. Observations were made before, during and following the administration of the dye.

General observations and the initial appearance of the dye in the neurohypophyseal tissues in intact animals. Very soon after the beginning of the injection, the dye appears as a very thin, barely detectable, blue line immediately adjacent to some of the capillaries in the median eminence and stalk. Since the dye does not appear in all areas of the visible portions of the neurohypophyseal tissue at the same time, or in the same initial concentration, staining appears at first to be patchy and irregular. As the injection proceeds, the initial thin blue lines take on a deeper colour, gradually take on a granular appearance, and finally fill all the tissue between the capillaries. Finally, after the injection of a total dose of 0·01–0·02 c.c./g of body-weight, the stalk and median eminence are usually uniformly stained. If an area appears to be unstained or considerably more lightly stained than adjacent areas in an animal in which no deliberate cutting of the arterial vessels has taken place such an area has always been observed to have a reduced rate of blood flow in the capillaries. Animals in which several areas of markedly reduced flow were observed before the injection of methylene blue were seldom used for interruption of blood vessels although they can be mapped and their relation to the areas which are affected by manipulation can be determined (Fig. 2).

With the concentration of dye used, the neurohypophysis is the only part of the area under observation which is fairly intensely stained. The surrounding hypothalamic tissue is unstained to all practical purposes, and a small amount of the dye may be found along the anterior edge of the anterior lobe. The continued injection of dye in excess of the dose described above gives a gradually deepening colour to the median eminence and stalk; the neurohypophyseal area becomes blue-black, and the anterior margins of the anterior lobe become considerably more heavily stained. The effect of excess amounts of dye solution given intravenously is finally to interfere with the circulation in the hypophyseal portal system. The capillaries become clogged, and the portal blood flow slows over a period of time and finally stops with the only remaining detectable blood flow occurring in the larger arterial vessels around the stalk. An excessive dose of methylene blue was not routinely given.

If the injection of the dye is discontinued and the stained area observed for a period of time thereafter there is gradual fading of the blue colour. Whether this is due to a diffusion of the dye out of the stalk or the breakdown of the dye to a colourless form is not known. Evidently some diffusion of the dye takes place, but this does not occur so rapidly that small well-delineated vascular areas in the stalk cannot be observed by this technique.

Observation on excised specimens of hypothalamus and neurohypophysis following dye injections. In order to determine something of the more precise localization of the dye in the neurohypophyseal tissue, some specimens were excised following injection of varying amounts of the dye. These were observed *in vitro* with a water immersion lens without the application of a cover slip in order to maintain the dye in the oxidized (coloured) state. The information which has been derived from such observations is primarily negative. Very little can be determined about the nature of the dye and its distribution since, after the tissue has been excised, there is apparently acceleration of the diffusion process. In the living animal the dye appears granular at the levels of magnification obtainable (100–150 times). It also appears to lie rather superficially in the median eminence and stalk between the capillaries. So far it has been impossible to devise a method for obtaining a sufficiently well-preserved cross-section of the unfixed stalk to be able to describe accurately the localization of the dye in the deeper regions. The significant negative observation is that in no specimen that we have examined (either those described in this article or others) have we seen any staining of the small unmyelinated nerve fibres of the hypothalamico-hypophyseal tracts by methylene blue.

This negative evidence is strengthened considerably by the fact that in many of our preparations we have seen numbers of irregularly distributed nerve fibres in the hypothalamus as well as in and just behind the optic chiasma which are very dramatically stained by methylene blue. Occasionally I have seen fibres which are presumed to be vasomotor fibres passing along the courses of the hypophyseal and hypothalamic arteries and on numbers of occasions nerve fibres lying superficially in the hypothalamus (which must be either within the pia or immediately sub-pial) were seen passing into the median eminence and stalk. This was quite dramatically demonstrated when small blood vessels to the primary capillary plexus were interrupted leaving an unstained area in the middle of the stalk. One or two nerve fibres could be seen standing out against a light background. It should be emphasized that these fibres are not the well-organized fibres of the hypothalamico-hypophyseal tracts which stain with silver stains.

Interruption of arterial vessels of the median eminence and stalk. The arterial vessels around the median eminence and stalk and portions of the hypothalamus as they appear in the living animal have been described previously[4,5]. An excellent recent description of injected material has been given by Enemar[17]. The courses of the vessels will not be described in further detail here except where such a description relates directly to the delineation of vascular fields by the methylene blue technique. The result of cutting a small arteriole to the median eminence is shown in Fig. 1. Little or no dye appears in a small fairly well-delineated field. It must be emphasized at the outset that there is no vascular stasis in this small field. Blood continues to flow through the capillaries but at a markedly reduced rate when compared with other capillaries outside of the field. The line of demarcation between stained and unstained tissue is usually quite sharp and falls between capillaries of separate groups. Since no other direct arteriolar supply to this small unstained area can be observed, it is deduced that a reduced capillary blood flow in the area is maintained by anastomoses with surrounding capillaries[4]. It is not impossible that even smaller areas than that shown in Fig. 1 might be affected by this procedure if there were not a limit on the size and location of arteriole which may be interrupted by freehand methods. A larger area of involvement is demonstrated in Fig. 2 after the interruption of a more proximal arterial vessel. Animals in which vessels were removed from one side of the stalk and median eminence are illustrated in Fig. 3. The demarcation between the stained and unstained areas roughly bisect the stalk.

Our experiments using this method suggest very strongly that the blood supply of the upper part of the stalk and median eminence are relatively independent of that of the lower stalk. The lower stalk must have its arterial supply intact to receive dye from the general circulation in sufficient concentration for it to appear in the tissue. The result of interrupting vessels to the upper and lower stalk

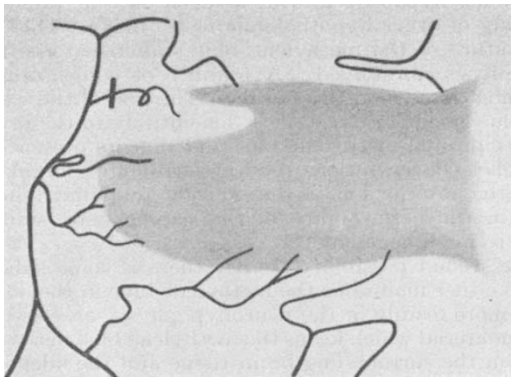

Fig. 1. Arteries and arterioles of the median eminence and stalk of the living mouse. The shaded area outlines the area of distribution of methylene blue in this region. The bar indicates the point at which an arteriole has been cut and the light area, the field in which no dye appeared although blood continued to flow through its capillary bed at a reduced rate. Artist's diagram from author's sketch of the living preparation

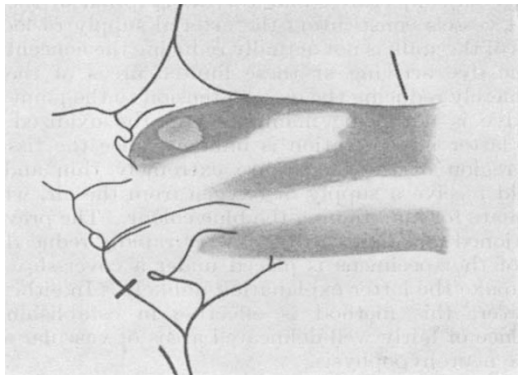

Fig. 2. Similar preparation to Fig. 1. More proximal branch of an artery cut as indicated by the bar. There is a much larger area of involvement. The blood flowed at a reduced rate through much of the lighter stained area. The oval area in the upper left part of the diagram represents a less well-stained area in which there was reduced blood flow before cutting stalk vessels

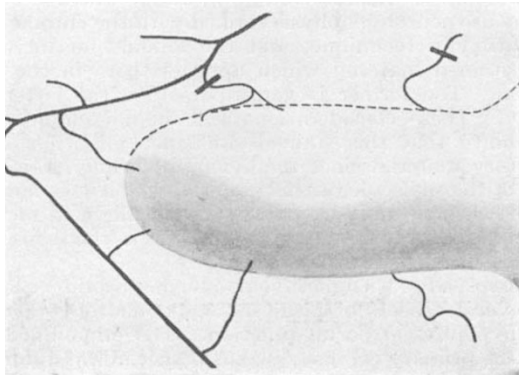

Fig. 3. Interruption of arteries of the left side of the median eminence and stalk

is illustrated in Figs. 4 and 5. The lower lateral margins of the stalk show no dye concentration after cutting the vessel on either side supplying this area (Fig. 4). If the reverse procedure is carried out and these vessels are left intact and others in the upper part of the stalk cut, something of the reverse of this picture is observed (Fig. 5). When only the vessels in the lower part of the stalk (sometimes only a single vessel) are left intact and all the other vessels of the upper stalk and median eminence are cut, one observes considerably more interference with the circulation in the primary capillary system and portal trunks from the upper stalk and median eminence than when some of the vessels in the upper part of the stalk are left intact. Under the former conditions one may see stasis in primary capillaries and in one or more portal vessels. Fig. 6 illustrates the stalk of an animal in which all the hypophyseal arterial vessels in the region had been cut except one which was partially closed as a result of mechanical manipulation. Those areas which are stained still showed fairly rapid flow in the capillaries.

The dye, in order to reach the tissues in sufficient concentration to become visible, must arrive there by way of the arterial vessels and not by capillary anastomoses from the upper part of the stalk and median eminence or by diffusion through the walls of portal trunks. In prepara-

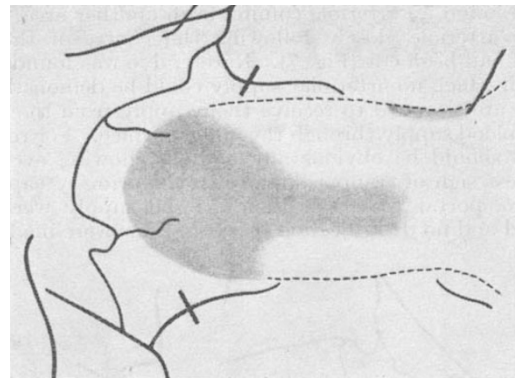

Fig. 4. Interruption of arteries to lower lateral areas of the stalk. Vessels to the upper stalk and median eminence intact

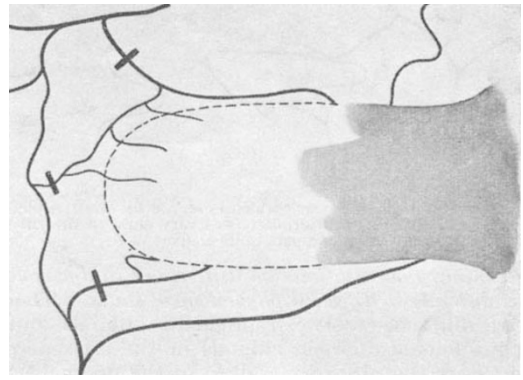

Fig. 5. Interruption of arteries and arterioles to the upper stalk and median eminence. Vessels to lower stalk intact

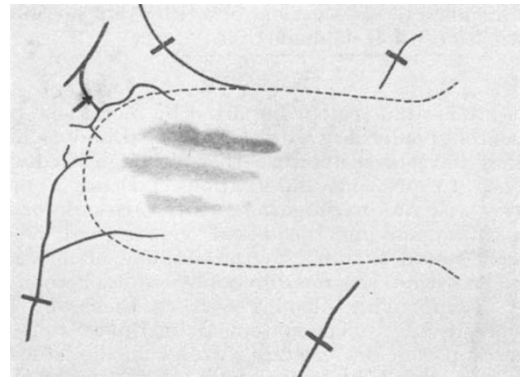

Fig. 6. All vessels to stalk and median eminence cut except that indicated by the arrow. The latter had been manipulated and was constricted, but rapid blood flow was still present. Fairly rapid capillary blood flow remained in the stained areas

tions in which a portal trunk traversed a lightly stained or unstained area the arterial supply of which was cut, no dye appeared in the area although both capillary anastomoses and the portal trunk passed from stained to unstained areas. Occasionally dye appears in an area from which we feel that we have removed the arterial supply or conversely a light area appears but no direct interference with the arterial supply has occurred.

These cases are infrequent. When it is a lighter area which was not anticipated (Fig. 2) there is always a reduced blood flow in this area. Whether this reduced flow is due to arteriolar constriction and reduction in rate of blood flow by physiologic mechanisms or through some incidental and unnoticed trauma during the procedure is not known. In the case of the appearance of dye in an area in which we thought we had removed the arteriolar supply, I afterwards found an arteriole coming from another area, or an intact arteriole closely following the course of the one which had been cut (Fig. 7). Rarely, dye was found in an area in which no arteriolar supply could be demonstrated. These are assumed to receive their supply from the lower stalk blood supply through the under surface. For reasons which should be obvious any animals showing even the slightest sign of trauma directly to the primary capillary system, portal vessels or their arterial supply were discarded and no dye injection investigations were made.

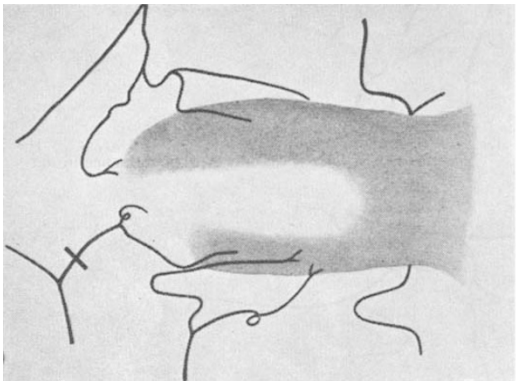

Fig. 7. Dye appears in an area which is served by an arteriole which has been cut. An intact arteriole passes very close to the cut vessel along part of its course

Preliminary observations on differences in dye concentration in animals in different physiological states. There is a distinct difference between pregnant animals and non-pregnant female or male animals in the amount of dye taken up by the pituitary stalk. In the pregnant female comparatively small amounts of the dye injected into the femoral vein impart an intense blue-black colour to the neurohypophysis much more rapidly than one finds in the non-pregnant female and the male animal. This is an incidental observation and thus far no intensive investigation has been made of it.

Discussion

The intense blue colour imparted by methylene blue to the neurohypophysis was observed by Dandy[15] in 1913 and may have been known earlier although he makes no reference to previous observations. Dandy's primary purpose was an investigation of the vasomotor nerve fibres accompanying the blood vessels and he quite correctly stated that he saw no staining of nerve fibres or tracts within the neurohypophysis other than those fibres accompanying blood vessels on their way to the anterior pituitary. The statement by Bucy[16] that it had become apparent since Dandy's work that the intense blue colour of the neurohypophysis is due to the presence of the many unmyelinated nerve fibres which are present is not supported by the observations reported in this article. In none of these were we able to find staining of the hypothalamico–hypophyseal tracts, even in the presence of staining of other hypothalamic nerve fibres. This leaves the nature of the methylene blue staining of the neurohypophysis unresolved. A number of conjectures may be made concerning this matter. The first of these is that the blue colour in the stalk is due entirely to the presence of precipitated methylene blue and that its presence here in higher concentrations than in surrounding brain tissue or in the anterior lobe is due to some fundamental difference in the permeability of the capillary or sinusoidal walls to methylene blue.

The second possibility is that there is some substance which either maintains the methylene blue in the oxidized state more readily in the neurohypophysis, or, conversely, some material which keeps the methylene blue in a reduced state in the surrounding brain tissue and the adenohypophysis. That there is present in the neurohypophysis a substance which maintains the dye in the oxidized state is doubtful. When these pituitary stalks are placed under cover-slips the oxygen is very rapidly used up and fading very rapidly occurs. The colour can be restored to the tissues when the cover-slip is removed and the tissue is allowed to come in contact with the air. In this connexion it may be conjectured that the interruption of the small blood vessels constituting the arterial supply to localized areas of the stalk is not actually reducing the concentration of the dye arriving at these limited areas of the stalk but merely reducing the oxygen tension to the point where the dye is not being maintained in the oxidized state. This latter interpretation is unlikely since the tissues in this region of the mouse are extremely thin and they should receive a supply of oxygen from the air, which is adequate for maintaining the blue colour. The previously mentioned fact that the dye is very rapidly reduced when one of the specimens is placed under a cover-slip would also make the latter explanation unlikely. In either case, however, this method is effective in establishing the presence of fairly well-delineated areas of vascular supply in the neurohypophysis.

Still another possibility concerning the nature of the staining reaction is that there is a specific affinity for some material in the neurohypophysis for the methylene blue. The first material which should be considered is neurosecretory material in the classic Scharrer sense[18]. This seems unlikely since there is a marked discrepancy between the amount of neurosecretory material which occurs in the mouse neurohypophysis stained with the chrome–alum hæmatoxylin technique, and the amount of methylene blue stained material which appears there in the living animal. The former is very sparse[19,20], the latter very heavy. These considerations still do not eliminate the possibility that this stained substance might be neurosecretory material since methylene blue may stain some part of the molecule of this material which does not stain by the chrome–alum hæmatoxylin and might in fact be a better stain for neurosecretory material in the mouse than chrome–alum hæmatoxylin.

These studies with methylene blue have a direct bearing on several aspects of pituitary stalk, neurohypophyseal, and hypophyseal portal function. The unmodified view that the primary capillary plexus is specialized in function only by virtue of location and vascular connexions pervades the literature on pituitary–hypothalamic relationships. Anatomical evidence for distinct groupings of capillaries in man and mouse was mentioned in the introductory paragraphs of this report.

The experiments reported here show that the pituitary stalk and median eminence can be sub-divided, on a vascular basis, into various areas which are functionally separate although there are capillary anastomoses between them. A sufficient rate of perfusion of blood through the capillaries of these areas by way of arterioles must be maintained for a uniform concentration of methylene blue in the blood to diffuse into the neurohypophyseal tissue. It is not an unreasonable hypothesis that this

might be true of other materials as well. These experiments show further that the upper and lower parts of the stalk have a relatively independent vascular supply.

The demonstration of more than one adenohypophyseal trophic factor from the hypothalamus[11-13] leads one to the question whether these materials are being released uniformly throughout the median eminence and stalk, or whether, alternatively, certain functions are located in separate areas of the stalk. One may ask further, whether the rate of release of these materials is governed completely by some functional attribute of the neuron or neuron terminals from which they are released or whether variations in the rate of blood flow through independent vascular fields contributes something to the rate of delivery of these materials into the portal trunks and thence into the adenohypophysis.

Although any conclusion on these matters at the present time would be premature, the experiments reported here demonstrate that such functional areas could exist and could be controlled in some measure by vasomotor control of blood flow into a given area. Experiments demonstrating chronic insufficiency of specific stalk-mediated functions by interrupting vessels to specific areas and maintaining the animals for long periods of time have been attempted. They have all so far failed because animals have not survived the experiment. The factors involved are not entirely clear although it does not involve the interruption of hypothalamic blood vessels or damage to the hypothalamus or to the pituitary stalk. Sham-operated animals with no manipulation of stalk vessels or hypothalamic damage also failed to survive.

The second major point which these experiments demostrate is that 'titre' or concentration of material in the circulating blood is not the only factor controlling the rate at which materials can arrive in the median eminence and stalk or in the anterior lobe.

It is obvious from these experiments that rate of capillary blood flow markedly influences the ability of the neurohypophysis to take up and concentrate the dye methylene blue. In every case where there were lighter areas of the stalk after injection of the dye there was a reduced blood flow rate. It can be concluded that there was a sufficient concentration of the dye in the blood of the stalk capillaries flowing at a rate insufficient to bring about a visible accumulation in the tissues.

In summary, these experiments with a dye which diffuses readily from primary capillaries into the neurohypophyseal tissue lends support to the following hypotheses: (1) That small regions of the pituitary stalk and median eminence are functionally separable on the basis of the arterial supply to different parts of the primary capillary system. (2) That capillary perfusion rate is an important factor in determining the amounts of materials which pass from primary capillaries to neurohypophyseal tissue. (3) That the arterial blood supply to the upper stalk and median eminence is functionally relatively independent of the lower stalk. (4) That the permeability of the primary capillaries varies with physiological state. It is possible that all these factors are involved in the fine control of pituitary function by hypothalamic neurohumours and/or materials from the general circulation.

This work was supported by U. S. Public Health Service grants *B*–3937 and *H*–4176

[1] Harris, G. W., in *Handbook of Physiology*. Section 1: *Neurophysiology*, **2**, edit. by Field, J., Magoun, H. W., and Hall, V. E., 1007 (American Physiological Society, Washington, 1960).
[2] *Proc. Neuroendocrinol. Symp., Miami, Florida*, December 1961 (to be published).
[3] Worthington, jun., W. C., in *Blood and Lymph Vessels*, edit. by Abramson, D. I., 428 (Academic Press, New York, 1962).
[4] Worthington, jun., W. C., *Bull. Johns Hopkins Hosp.*, **97**, 343 (1955).
[5] Worthington, jun., W. C., *Endocrinol.*, **66**, 19 (1960).
[6] Goldman, H., and Sapirstein, L. A., *Amer. J. Physiol.*, **194**, 433 (1958).
[7] Goldman, H., *J. App. Physiol.*, **16**, 762 (1961).
[8] Fumagalli, Z., *Z. Anat. Entwicklungsgeschichte* (Berlin), **111**, 266 (1941).
[9] Green, J. D., *Anat. Rec.*, **100**, 273 (1948).
[10] Xuereb, G. P., Prichard, M. M. L., and Daniel, P. M., *Quart. J. Exp. Physiol.*, **39**, 219 (1954).
[11] McConnell, E. M., *Anat. Rec.*, **115**, 175 (1953).
[12] Guillemin, R., Schally, A., Andersen, R., Lipscomb, H., and Long, J., *C.R. Acad. Sci. Paris*, **250** (3), 4462 (1960).
[13] McCann, S. M., Tabeisnik, S., and Friedman, H. M., *Proc. Soc. Exp. Biol. Med.*, **104**, 432 (1960).
[14] Haselbach, C. H., and Libert, O., *Acta Endocrinol.*, Supp. 67, 57 (1962).
[15] Dandy, W. E., *Amer. J. Anat.*, **15**, 333 (1913).
[16] Bucy, P. C., in *Cytology and Cellular Pathology of the Nervous System*, edit. by Penfield, W., **2**, 705 (Paul B. Hoeber, Inc., New York, 1932).
[17] Enemar, A., *Arkiv. Zool.*, Ser. 2, **13**, 203 (1961).
[18] Scharrer, E., and Scharrer, B., *Rec. Prog. Hormone Res.*, **10**, 183 (1954).
[19] Becker, H., *Deutsch Z. Nervenheilkunde*, **173**, 123 (1955).
[20] Worthington, jun., W. C. (unpublished observations).

A WATER-SOLUBLE POLYPEPTIDE PREPARED FROM ZYMOSAN

By P. LALAND
Nyegaard and Co., A/S, Oslo
J. DEDICHEN
Rikshospitalet, University of Oslo
PROF. S. LALAND
Department of Biochemistry, University of Oslo, Blindern
R. OFTEBRO
Norsk Hydro's Institute for Cancer Research, Oslo
N. THORSDALEN
Nyegaard and Co., A/S, Oslo
AND
J. VOSS
State Institute of Public Health, Oslo

ZYMOSAN, an insoluble lipoprotein-polysaccharide derived from the cell wall of yeast cells, inactivates the third component of complement[1,2], produces hyperplasia and hyperfunction of the reticuloendothelial system[3-7]. Furthermore, it increases the resistance of mice towards bacterial infections[8] and ionizing radiation[9]. It enhances the formation of antibody[5] and also prevents the development of tumours[10] in animals and is pyrogenic and antigenic in rabbit[11].

This article describes a method for producing from zymosan a water-soluble polypeptide. This material protects, after intraperitoneal injection, mice against *E. coli* infection. Furthermore, it diminishes the lesion produced by vaccinia virus in rabbit skin. It also provides some protection of chick embryo against vaccinia virus. Finally, in tissue culture it inhibits the mitotic activity of HeLa cells.

2 g of commercial zymosan (from fresh yeast, type *A*, L. Light and Co., Colnbrook, England) prepared from Fleischmann yeast was extracted at 37° C with 150 ml. of 90 per cent aqueous phenol for 2 days. Undissolved material was removed by centrifuging and the clear