THE RAPID INVASION OF THE BODY THROUGH THE OLFACTORY MUCOSA

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PLATES 8 TO 10

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Clark (1) in 1929 described the introduction of Prussian blue into the nasal cavities of rabbits to determine the routes by which infectious agents pass from these cavities to the brain. At the earliest time of study, 1 hour after nasal inoculation of the pigment, granules were found between the cells of the olfactory mucosa, but not the respiratory, in the submucosa, the lymphatics and venules, in the perineural sheaths and between the fibers of the olfactory nerve, but not the trigeminal, and within the subarachnoid space about the olfactory bulbs. He demonstrated that the perineural spaces of the olfactory nerve are continuous with the subarachnoid space and considered that the granules had reached the olfactory bulbs mainly by passage along the perineural spaces. To account for this migration he postulated a centripetal current in these spaces. He found no evidence of direct communication between the perineural or subarachnoid spaces and the nasal lymphatics and did not determine the method by which the pigment penetrates the mucosa nor the shortest interval in which granules can reach the intracranial cavity.

These essential findings have been confirmed by Olitsky and Cox (2) in experiments with mice. Moreover we (3, 4) have shown that pneumococci instilled intranasally in mice pass rapidly through the mucosal surfaces, including those of the nose, to reach the peripheral blood within 2 minutes. We also, in repeating the tests with Prussian blue in mice, have found (5) that absorption is very rapid, taking place chiefly by way of the olfactory cells, and that within 2 minutes pigment has passed into lymphatics and blood vessels, into perineural

sheaths and between nerve fibers and thence to the subarachnoid space and pia mater over the olfactory bulb. These latter findings, together with similar tests with bacteria and viruses, are described in the present paper.

Passage of Prussian Blue through the Olfactory Mucosa

10 per cent solutions of iron ammonium citrate and potassium ferrocyanide are prepared before each experiment. Equal amounts are mixed and the resulting particulate suspension is allowed to fall in small droplets on the outside of the nostrils whence it is breathed into the nose. In this manner 0.02 cc. is administered to each mouse—0.01 cc. in each nostril—without trauma or increased pressure within the nose. The mice are decapitated at intervals after inoculation. The head, without lower jaw and tongue, is placed in 10 per cent formalin containing 5 per cent HCl which acts as a decalcifying agent and also precipitates the pigment. After 5 days the heads are removed and divided sagittally along the midline. The halves are embedded in paraffin. Sections are prepared, lightly stained with Mayer's carmine and mounted.

Three experiments have been carried out. In the first, 20 Swiss mice were treated and 2 were decapitated at 2, 5, 15 and 30 minutes, 1, 2, 4, 6 and 8 hours. In a second test 4 mice of a strain susceptible both to bacterial and virus infections and 4 mice of a strain resistant to both types of infection were used (6), 2 of each group being sacrificed at 2 and 15 minutes. In the third test 12 mice of a bacteria-susceptible-virus-resistant strain, and 12 of a bacteria-resistant-virus-susceptible strain were used (6), 6 of each group being killed at 2 and 15 minutes after inoculation.

In the first experiment with 20 Swiss mice, the 2 killed within 2 minutes of inoculation showed widespread distribution of the dye. The picture seen at 1, 2 and 3 hours after inoculation (1, 2) is one only of end-results.

2 Minutes.—The pigment lies thickly spread over the surface of both olfactory and respiratory (ciliated) epithelium and is mixed with mucus, the latter being in smaller amounts than are found later.

The granules are passing through the olfactory mucosa. On first glance they appear to lie in narrow channels (Fig. 1) between the supporting cells, but on closer examination these streams of pigment are found to be clustered around the fibrils of the olfactory neurones which run to the free surface of the mucosa. Furthermore the granules fill the cystoplasm, but not the nucleus, of the neurone (Figs. 2, 3, 4). In some cases, moreover, the whole cytoplasm, but never the nucleus,

besides containing granules, is stained a diffuse blue. The streams of pigment can never be traced into the zone of mucosa next to the basement membrane, *i.e.*, below the layer of neurones, where they should appear if passing in intercellular channels. Thus while scattered granules may pass in between the cells, the greater part appear to travel through the olfactory cells themselves.

Over the respiratory mucosa the granules are often thickly tangled in the cilia. Some ciliated cells are stained diffusely blue and the nuclei are filled with granules. Such cells appear to be dead or damaged. Granules also appear in the cytoplasm of healthy ciliated cells or between the bases of such cells, showing that absorption is taking place through and between the respiratory cells. No absorption occurs through squamous epithelium.

The crypts of the serous glands contain pigment, and fine granules can occasionally be seen in the cytoplasm of the secreting cells.

In the submucosa the granules are scattered widespread in tissue interstices or, infrequently, inside cells. For the most part they line, or even fill a network of ramifying thin walled channels with all the characteristics of lymphatics (Fig. 5). These channels are especially full below an area of mucosa where absorption is most active. They empty into larger channels or penetrate the cartilaginous septum. Capillaries and venules contain scattered granules, while sinusoids in the septum or turbinates often show many. Capillaries or small venules, accompanying branches of the olfactory or trigeminal nerve or running in separate foramina through the cribriform plate, contain scattered granules.

The perineural spaces of the small branches of the olfactory nerve, supplying areas of mucosa where absorption is most active, are filled with granules of Prussian blue and the pigment lies between the nerve fibres in the middle of the nerve (Fig. 6). This marked concentration of pigment is confined to these small branches. The terminal twigs supplying the mucosa show scattered granules along their length and the main branches which pierce the cribriform plate, while showing granules in the perineural sheath and between the fibres, contain much less than the small branches. A few granules adhere to the nerve as it crosses the subarachnoid space to the olfactory bulb but are never seen within the brain itself save, very seldom, inside the small vessels. Branches of the trigeminal nerve show granules between the fibrils, in the perineural space or outside the perineural sheath. The granules are far fewer than along the olfactory branches.

The most surprising feature is in the subarachnoid space and the pia-arachnoid membrane of the olfactory bulb. Even at this early time the arachnoid network in the anterior angle of the skull contains granules (Fig. 7) as does the pia-arachnoid membrane (Figs. 8 and 9). Sinuses beneath the dura, especially the cavernous, contain much pigment.

5 Minutes.—Absorption is still active through both the olfactory and the respiratory mucosae but is less than at 2 minutes. The amount of mucus on the surface has increased. In the submucosa the lymphatics are often filled and the blood vessels contain more pigment than before. Pigment has decreased in the

small branches of the olfactory nerve but in the main branches the granules are as frequent as at 2 minutes, though the majority now lie in the perineural sheath rather than between the fibres. Some pigment is still to be seen in small branches of the trigeminal nerve. In the subarachnoid space the granules are as plentiful as at 2 minutes but fewer are seen in the pia-arachnoid membrane.

15 Minutes.—Absorption through the olfactory mucosa is considerably less. Through the respiratory mucosa it has almost ceased. Everywhere in the submucosa, save in the sinusoids of the turbinates, the pigment has greatly diminished. A few branches of the olfactory nerve contain as much pigment as at 2 minutes, but for the most part the pigment occurs only in scattered clumps in the perineural sheath. Granules are scattered in the subarachnoid space as far posteriorly as the pituitary fossa, and over the dorsum of the cerebrum. A few clumps are still present in the pia-arachnoid membrane.

From now on there is a steady decrease of pigment everywhere in the tissues although some absorption is still taking place.

8 Hours.—This is the longest time interval prior to examination. Very slight absorption is still taking place through and between the olfactory neurones. Masses of pigment, entangled in mucus, lie over the mucosa. In the submucosa a very few granules appear in the interstices or in lymphatics below areas of mucosa where absorption is still occurring. A few branches of the olfactory nerve show small clumps of pigment in the perineural sheath and similar clumps appear in the subarachnoid space over the olfactory area and anterior cerebrum. Scattered granules are still present in the pia-arachnoid over the tip of the olfactory bulb.

The Swiss mice used for the above test are very susceptible to certain bacteria and viruses given intranasally. Breeds of mice varying in their innate resistance to these bacteria and viruses were available in the laboratory (6) and were tested by the same technique. Previous results (4) had suggested that the innate difference in resistance in these breeds lay somewhere in the tissues rather than at the body surface.

Four BRVR (bacteria- and virus-resistant) and 4 BSVS (bacteria- and virus-susceptible) were used. They were inoculated intranasally with 0.02 cc. of 10 per cent Prussian blue by the technique described above. 2 of each group were decapitated at 2 minutes and 2 at 15 minutes after inoculation. Sections were prepared exactly as in the Swiss mice.

2 Minutes.—The picture in the BSVS mice resembles that described at 2 minutes in the Swiss. The BRVR mice show certain quantitative differences in that there are fewer granules both within the perineural sheath of the small branches of the olfactory nerve and in the subarachnoid space and pia-arachnoid membrane.

15 Minutes.—Again the picture in the BSVS mice resembles that seen at 15

minutes in the Swiss. The BRVR mice, however, show considerably more in the small branches of the olfactory nerve and in the intracranial cavity. Indeed the picture resembles that seen in the Swiss and BSVS animals at 2 minutes.

It appears, therefore, that both susceptible breeds respond alike. In the resistant animals, however, passage into the tissues and vessels occurs as rapidly as in the susceptibles, but passage into the nerves and thence to the subarachnoid space is slightly delayed. This observation was made with only few mice and its significance is doubtful.

When the experiment was repeated with BRVS and BSVR mice (12 in each group and 6 of each sacrificed at 2 minutes and 6 at 15 minutes) no difference could be found in either group from the picture described in the Swiss and BSVS mice.

Olitsky and Cox (2) found absorption of pigment less evident in tannic acid-treated mice than in normal ones studied 1 and 3 hours after inoculation of Prussian blue. We repeated the experiments and examined mice within a few minutes after instilling the dye. The results have been reported briefly elsewhere (7).

Eight treatments of 0.03 cc. of 0.8 per cent tannic acid in 1 per cent glycerine were given during 3 days to 9 Swiss mice. The mice were given intranasally 0.02 cc. of the Prussian blue mixture 4 hours after the last tannic acid treatment. 5 mice were sacrificed 2 minutes and 4 mice 15 minutes later. Microscopical preparations were made in the way described above. Most were stained with Mayer's carmine; a few with hematoxylin and eosin.

Examination revealed two changes from the picture seen in untreated mice. First, although tannic acid-treated mice rarely show any exudate from the nose during life, actually, the nasal cavities over the olfactory mucosa and around the turbinates were filled with a heavy exudate of polymorphonuclear cells lying in thick mucus. The mucosa itself and submucosa were infiltrated with fluid and cells; the capillaries in the submucosa were dilated and contained many leucocytes.[1] The respiratory mucosa showed little change.

The absorption of pigment at 2 and 15 minutes was only slightly less than in untreated mice. Granules were as plentiful in the tissue spaces and in the vessels, but there was quantitatively less pigment

[1] The inflammation occurs when 1 per cent tannic acid in normal saline is used and is due therefore to the tannic acid rather than the glycerine.

along the branches of the olfactory nerve, in the subarachnoid space and in the pia-arachnoid than is seen in untreated mice at these times.

The second striking departure was found in the olfactory mucosa. In the tannic acid-treated mice the selective passage of pigment into the olfactory neurones was not seen. In 6 of the 9 mice these cells contained no granules and in 3 only a few olfactory neurones contained granules. The 6 mice showing no pigment in these neurones were those with the most intense inflammation. In the other 3 the inflammation was less. A causal relationship between the inflammation and the absence of passage of pigment into the neurones has not been shown; it seems more probable that both departures from the normal are parallel responses to the tannic acid.

The above studies show that pigment instilled intranasally in the mouse is absorbed very rapidly. Absorption occurs chiefly through the olfactory mucosa where the pigment selects the olfactory neurones. Within 2 minutes pigment is present inside vessels and within the perineural sheath of the olfactory nerve along which it has reached the subarachnoid space and pia mater. Subsequently it slowly disappears. Absorption of pigment is not prevented by preliminary tannic acid treatment. Such treatment does decrease the amount of pigment reaching the perineural space, the subarachnoid space and the pia mater, but its principal actions are to cause an acute inflammation of the olfactory mucosa and an almost complete interruption of the passage of pigment into the olfactory neurones.

Passage of Bacteria through the Olfactory Mucosa

It has been shown (4) that pneumococci instilled intranasally in mice appear very rapidly in the peripheral blood. The results suggested that this invasion occurred both through the alveoli of the lung and also through the olfactory mucosa.

It was decided to investigate further the distribution of pneumococci in the body in the early periods after intranasal instillation. If they behave like pigment, and if invasion occurs through the olfactory mucosa, one should obtain early positive cultures from the olfactory area of the brain even in the absence of positive cultures in blood and other organs.

Mice were given 0.02 cc. of a 16 hour broth culture of intranasally virulent Type III pneumococci by the usual technique. At given times after inoculation they were sacrificed and various tissues cultured for pneumococci. In removing the brain, from which only the olfactory area was cultured, great care was taken to avoid opening the nasal cavity from above. The results of cultures from blood, spleen and olfactory area, in 4 tests using 31 mice sacrificed at periods up to 30 minutes, are shown in Table I. During this time 6 mice showed positive blood or spleen cultures and 23 positive olfactory area cultures.

The experiment was repeated using *Salmonella enteritidis*. 2 tests with 51 mice were carried out. Blood and olfactory area were cultured for bacteria at intervals up to 30 minutes. From Table I it will

TABLE I

Organism	Organ	Time in minutes													
		1	2	3	4	5	6	8	10	12	15	20	22½	25	30
Pneumococci	Blood and/or spleen	0/4*	2/4			0/4		0/4	1/5		0/2	2/3	0/1		1/4
	Olfactory area of brain	3/4	2/4			3/4		3/4	5/5		0/2	3/3	1/1		3/4
B. enteritidis	Blood	0/3	1/3	1/3	0/3	0/3	0/3	0/3	1/6	0/3	1/6	2/6		0/3	0/6
	Olfactory area of brain	1/3	2/3	1/3	1/3	1/3	1/3	0/3	4/6	2/3	2/6	3/6		2/3	4/6

* 0/4 = no positive cultures out of 4 taken at this time interval.

be seen that 6 mice gave positive blood cultures and 24 positive olfactory area cultures.

Both a respiratory and an intestinal pathogen, therefore, can reach the brain shortly after contact with the olfactory mucosa. The 4:1 preponderance of positive olfactory area cultures over those obtained from the blood or spleen seems to indicate that the former are not due to bacteremia.

Studies were made[2] to determine whether tannic acid in dilutions effective against virus diseases (2) could protect mice against intranasal instillation of pneumococci. The results reported elsewhere (8) show that such preliminary treatment gave no protection against pneumococci. During these studies it was found that the pneu-

[2] These studies were made with Dr. H. R. Cox as collaborator.

mococci reached the blood as rapidly in the tannic acid-treated mice as they did in the untreated animals. Subsequently we have found that the pneumococci reach the olfactory area as rapidly in treated mice as they do in untreated. The tannic acid treatment therefore does not influence the passage of pneumococci through the olfactory mucosa.

In order to study the actual passage of pneumococci through the mucosa 4 mice were each given by the usual technique 0.03 cc. of a 12 hour broth culture of Type III pneumococcus, centrifuged down and resuspended in one-third the original volume. 2 minutes later all mice were decapitated. Fixation and decalcification was done in 10 per cent Zenker's acetate fluid. Sections were prepared as above but stained with Giemsa's stain.

Microscopically pneumococci are seen lying on the surface of the mucosa between fibrils or cilia. In the olfactory region some lie between cells near the surface but the majority lie between supporting cells close to the basement membrane. All invasion seems to be between the cells. In the submucosa pneumococci are seen in the tissue spaces, some inside phagocytes, in the lymphatics and, to a lesser degree, in the capillaries. They also appear in the perineural space of the olfactory but not the trigeminal nerve and are frequent in the subarachnoid space and within cells of the pia mater. Invasion between cells of the respiratory mucosa occurs but is considerably less.

Distribution tests and direct microscopical examination show therefore that pneumococci and *S. enteritidis* enter the body through the olfactory mucosa very rapidly and, like the pigment, immediately reach the intracranial cavity. Passage through the mucosa is accomplished between the cells and not by way of the olfactory neurones. This passage is in no way hindered by preliminary tannic acid treatment.

Passage of Viruses through the Olfactory Mucosa

Following the results with Prussian blue and bacteria the behavior of viruses inoculated by the same technique has been studied. Tests have been carried out with four viruses (Table II).

Mice are given 0.03 cc. of a suspension of the test virus intranasally by the usual technique. Virus suspensions are prepared in hormone broth usually at a dilution of 1:5 but in 2 cases at 1:10 and 1:100. At given times after inoculation the mice are killed and the tissues tested for virus. Usually the olfactory area of the brain and the spleen are tested. In 2 cases the spleen was omitted and in

TABLE II

Virus used and dilution	Organ tested	Minutes										Hours			Days		
		1	2	3	4	5	6	7	8	10	30	1	3	6	1	2	3
St. Louis encephalitis 1/10	Olfactory area		0, 0							0, 0	0, 0	0, 0	0, 0		5, 6	5, 8	
St. Louis encephalitis 1/100	" "		0, 0							0, 0	0, 0	0, 0	0, 0		0, 0	4, 5	
St. Louis encephalitis 1/5	Spleen		0, 0	0, 0		0, 0	0, 0	0, 0							6, 11		
	Olfactory area		†, 0	0, 0		0, 0	0, 0	0, 0							7, 0		
Louping ill 1/5	Spleen		0, 0		0, 0		0, 0		0, 0	0, †		0, 0	0, 0		0, 0		0, 0
	Olfactory area		0, 0		0, 0		0, 0		0, 0	0, 0		0, 0	†, 0		8, 8		8, 9
Louping ill 1/5	Spleen		0, 0		0, 0		0, 0		0, 0	0, 0		0, 0	†, 0		0, 0		0, †
	Olfactory area		0, 0		0, 0		0, 0		0, 0	0, 0		0, 0	0, 0		10, 13		8, †
Eastern equine encephalomyelitis* 1/5	Spleen	0, 0	0, 0		0, 0		0, 0		0, 0	0, 0				0, 0	0, 0		
	Olfactory area	0, 0	3, 3		3, 4		0, 0		0, 0	0, 0				0, 0	3, 3		
Eastern equine encephalomyelitis 1/5	Spleen + heart's blood	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0							0, 0	0, 0		
	Olfactory area	0, 0	2, 2	0, 0	2, 4	3, 4	0, 0							0, 0	2, 2		
Rabies 1/5	Spleen + heart's blood			0, 0	0, 0		0, 0		0, 0	0, 0							
	Olfactory area			0, 0	0, 0		0, 0		0, 0	0, 0							

2 mice injected intracerebrally with each test material. Blanks indicate that no test was made. 0 = mouse survived well. 3, 4, etc., = mouse died from the specific infection on the 3rd, 4th, etc., day. † = mouse died from trauma during inoculation.

* We are indebted to Dr. Olitsky and Dr. Cox for this virus. Prior to the early positive results which we found with Eastern equine encephalomyelitis virus, Dr. Cox had noted the same phenomenon occasionally (unpublished work).

3, half of the fluid used for dilution consisted of heart's blood from the same mouse, used to increase the possibility of finding small amounts of virus in the blood. The tissues are ground and suspended in hormone broth 1:10. 2 mice are inoculated with 0.03 cc. of each test suspension.

It will be seen that the viruses fall into two groups. St. Louis encephalitis, rabies and louping ill viruses[3] do not behave like pigment or bacteria but appear to be held up at the surface. The olfactory area of the brain is never positive until 24 hours after inoculation. Equine encephalomyelitis, however, resembles pigment and bacteria in reaching the intracranial cavity very rapidly. The fact that this virus is demonstrable only at 2 and 5 minutes after inoculation and then disappears is a further resemblance to the behavior of the pigment which reaches its maximum at 2 to 5 minutes. The virus reappears in the brain at 24 hours.

DISCUSSION

The above experiments suggest, *inter alia*, that the intranasal technique is more artificial than is usually supposed. By means of it substances are brought into widespread contact with the highly permeable surfaces in nose and lung (4) even when only 0.005 cc. is given. Such an exposure of mucosa does not occur normally in nature but represents, nevertheless, in exaggerated manner the slight mucosal contaminations which must be so frequent. Probably these normal contaminations are followed by rapid penetration of the mucosa, but their slightness and the resistance of the tissues limits the number of cases in which disease follows. That resistant hosts can deal with extensive preliminary invasion by bacteria is shown by the fact that mice resistant to Type III pneumococci given intranasally show as frequent early positive blood cultures as do susceptibles which die of the infection (4). During the present study it has been found that resistant mice show as frequent positive cultures from the olfactory area of the brain after intranasal instillation of pneumococci as do the susceptibles (Table III).

Pigment, bacteria and at least one virus given intranasally reach the tissues, the circulation and the brain very rapidly. In the last

[3] According to a test carried out by Dr. A. B. Sabin, vesicular stomatitis virus (New Jersey strain) belongs to this group (personal communication).

region the maximum concentration is reached in from 2 to 5 minutes. Pigment enters largely through the olfactory neurones but the bacteria appear to pass between the mucosal cells. The fact that tannic acid, which prevents the passage of pigment into the olfactory neurones, offers protection against certain viruses, suggests that the latter may enter these olfactory sensory cells as does the pigment.

The experimental results suggest an explanation of the fate of viruses instilled into the nose. If neurotropic viruses do enter the olfactory neurones like the pigment, they would tend rather to be held here than to pass freely to the tissues and the brain. Experimentally they have not been found in the brain immediately. The pantropic viruses,

TABLE III

	Organ	Time in minutes						
		1	2	5	7½	10	20	30
Resistant mice	Spleen	0	+	0	0	0	0	0
	Blood	0	+	0	0	0	0	0
	Olfactory lobe	+	+	+	0	+	+	+
Susceptible mice	Spleen	0	+	0	0	0	+	+
	Blood	0	+	0	0	0	+	+
	Olfactory lobe	+	+	+	+	+	+	+

0 indicates negative culture.
+ indicates positive culture.

however, should be able to pass freely out of the olfactory cells and experimentally one such virus has been shown to reach the brain immediately. This virus, equine encephalomyelitis, then disappears, probably being dispersed as is the pigment, and it reappears later. This disappearance may mean that the concentration is lowered below that needed to infect another mouse, but enough virus may remain to multiply and become redemonstrable later on. On the other hand the early invasion may be unimportant and may be effectively dealt with by the tissues, while the true infection progresses only slowly from the periphery. Slow progression seems to occur with the neurotropic viruses. It is still doubtful how this spread occurs, but it may be said that the evidence presented is contrary to Findlay's (9) and Hurst's (10) view of a slow progression in the perineural space.

SUMMARY

1. Prussian blue particles pass rapidly from the surface of the olfactory mucosa and within 2 minutes are found in the tissue spaces, in blood and lymph vessels, in the perineural spaces of the olfactory nerve fibers and in the subarachnoid space and pia-arachnoid membrane.

2. There is great affinity of pigment particles for the olfactory sensory cells.

3. Preliminary treatment of the olfactory mucosa with tannic acid does not alter the speed with which this absorption occurs. It does, however, cause an inflammation of the mucosa and appears to prevent the pigment from entering the olfactory sensory cells.

4. Both pneumococci and *S. enteritidis* pass through the olfactory mucosa and reach the tissue spaces, the vessels and the subarachnoid space with the same rapidity as the pigment. This can be demonstrated both microscopically and by distribution tests. They invade by passage between the cells of the mucosa and there is no apparent affinity of the organisms for the olfactory sensory cells.

5. Tannic acid treatment of the olfactory mucosa in no way alters this invasion of organisms through the mucosa.

6. The pantropic virus, equine encephalomyelitis, was detected in the forebrain as promptly as were pigment and bacteria; neurotropic viruses, however,—those of St. Louis encephalitis, rabies and louping ill,—were not demonstrated in less than 24 hours.

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EXPLANATION OF PLATES

PLATE 8

FIG. 1. 2 minutes. Mucous membrane. Pigment massed on the free surface of the olfactory mucosa and appearing to pass in streams between the superficial cells. In the submucosa pigment can be seen in the tissue interspaces. × 1000. Mayer's carmine.

FIGS. 2, 3 and 4. 2 minutes. Mucous membrane. In Figs. 3 and 4 the pigment is massed on the free surface of the olfactory mucosa. In all olfactory sensory neurones are shown with numerous pigment granules in their cytoplasm. Granules can be seen following along the sensory fibrils of 3 cells which run to the free surface. In Figs. 2 and 4 granules are seen in the submucosa. × 1000. Mayer's carmine.

PLATE 9

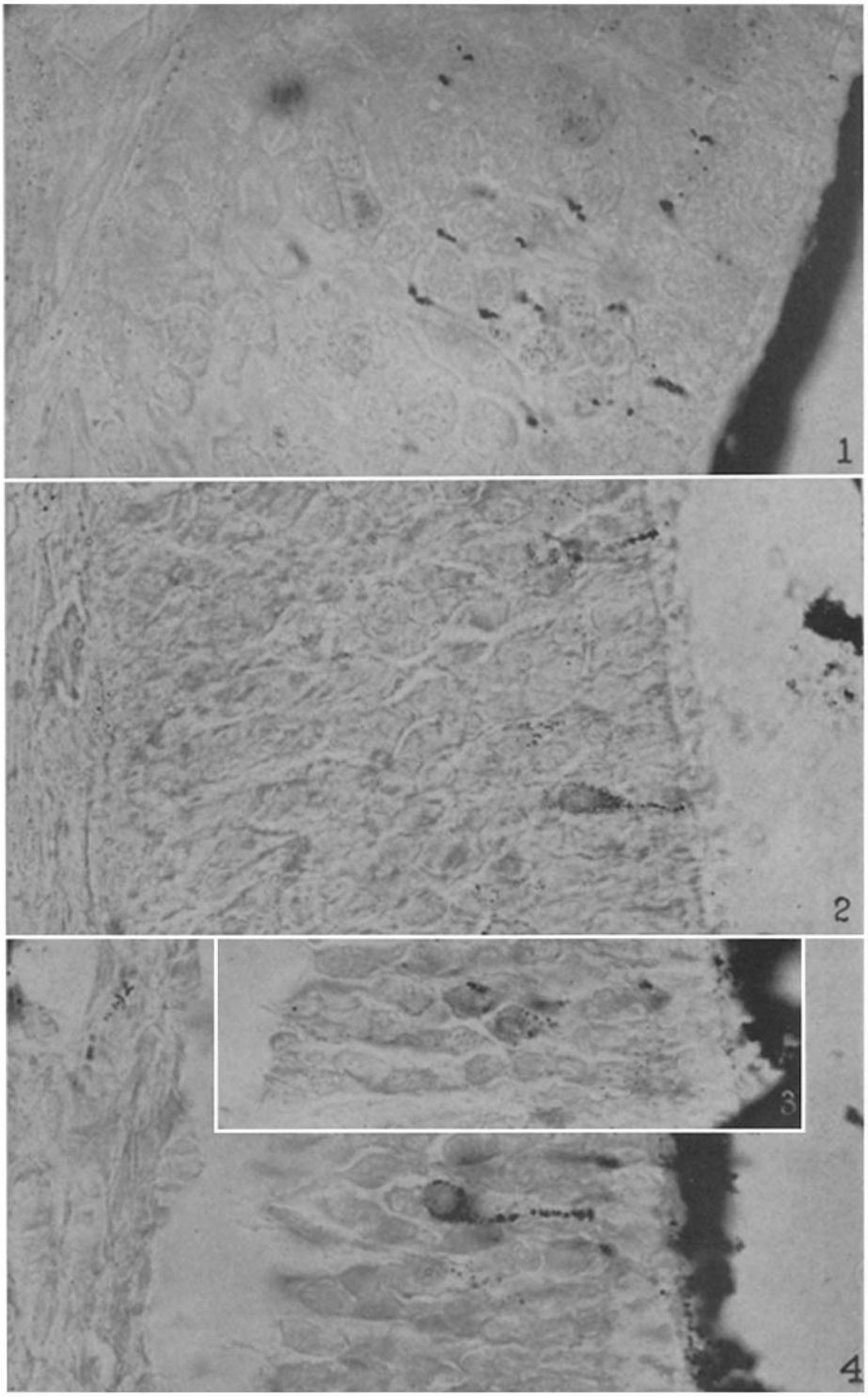
FIG. 5. 2 minutes. Submucosa. Ramifying lymphatic channels filled with granules. Some granules in larger blood vessels. To the right is a large branch of the olfactory nerve with pigment in the perineural space and between the fibres. × 400. Mayer's carmine.

FIG. 6. 2 minutes. Submucosa. A bifurcating branch of the olfactory nerve. The perineural space and the interfibral spaces are packed with Prussian blue granules. (This nerve appeared as a blue band to the low power of the microscope.) Above is a venule with pigment granules lining the endothelium. × 1000. Mayer's carmine.

PLATE 10

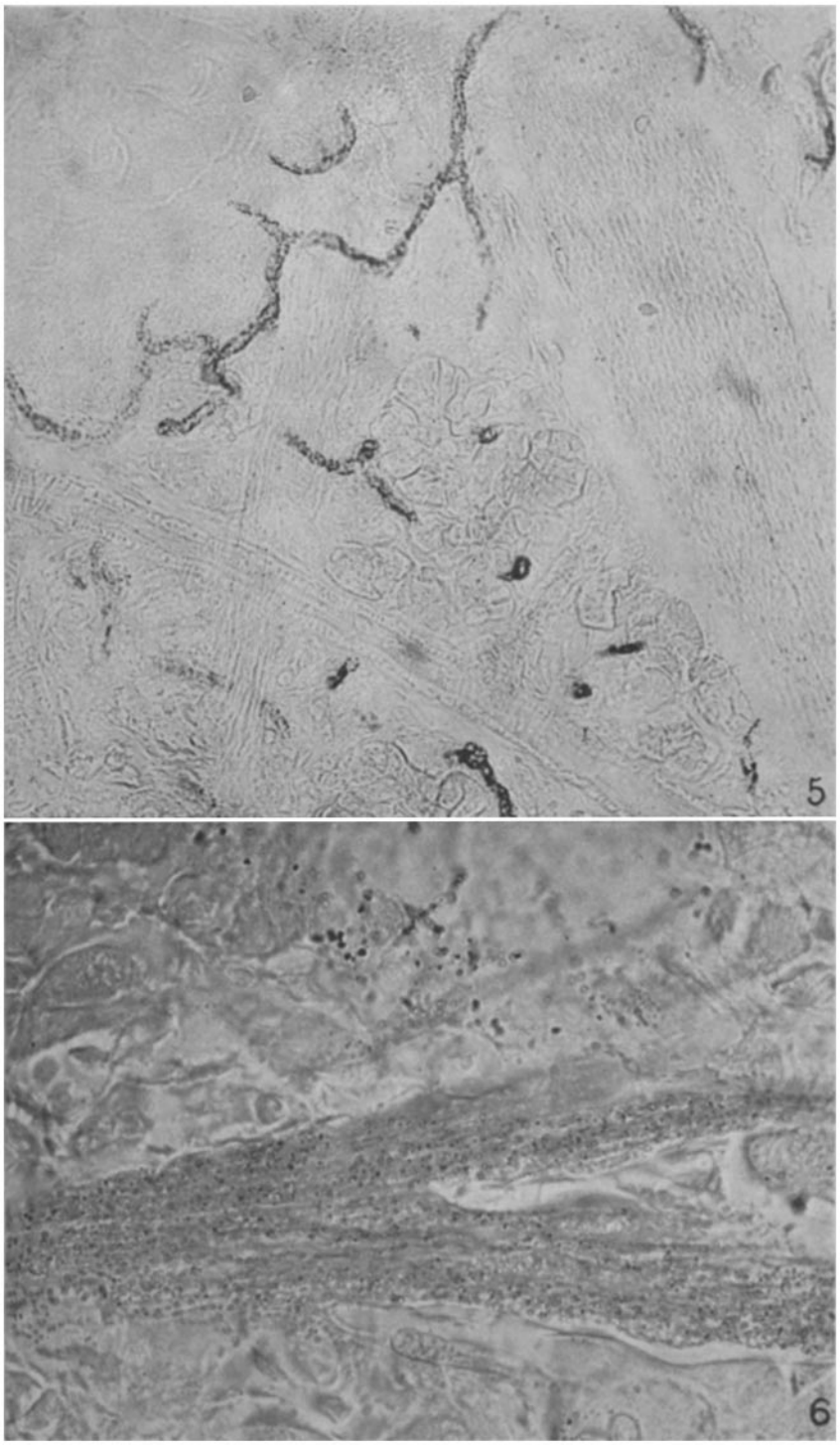
FIG. 7. 2 minutes. Subarachnoid space. Pigment granules, scattered or in masses, occupying the subarachnoid network at the anterior angle of the skull. × 1000. Mayer's carmine.

FIGS. 8 and 9. 2 minutes. Pia mater. Pigment granules in and between the cells of the pia over the olfactory bulb. None in the substance of the brain which lies below in both figures. × 1000. Mayer's carmine.



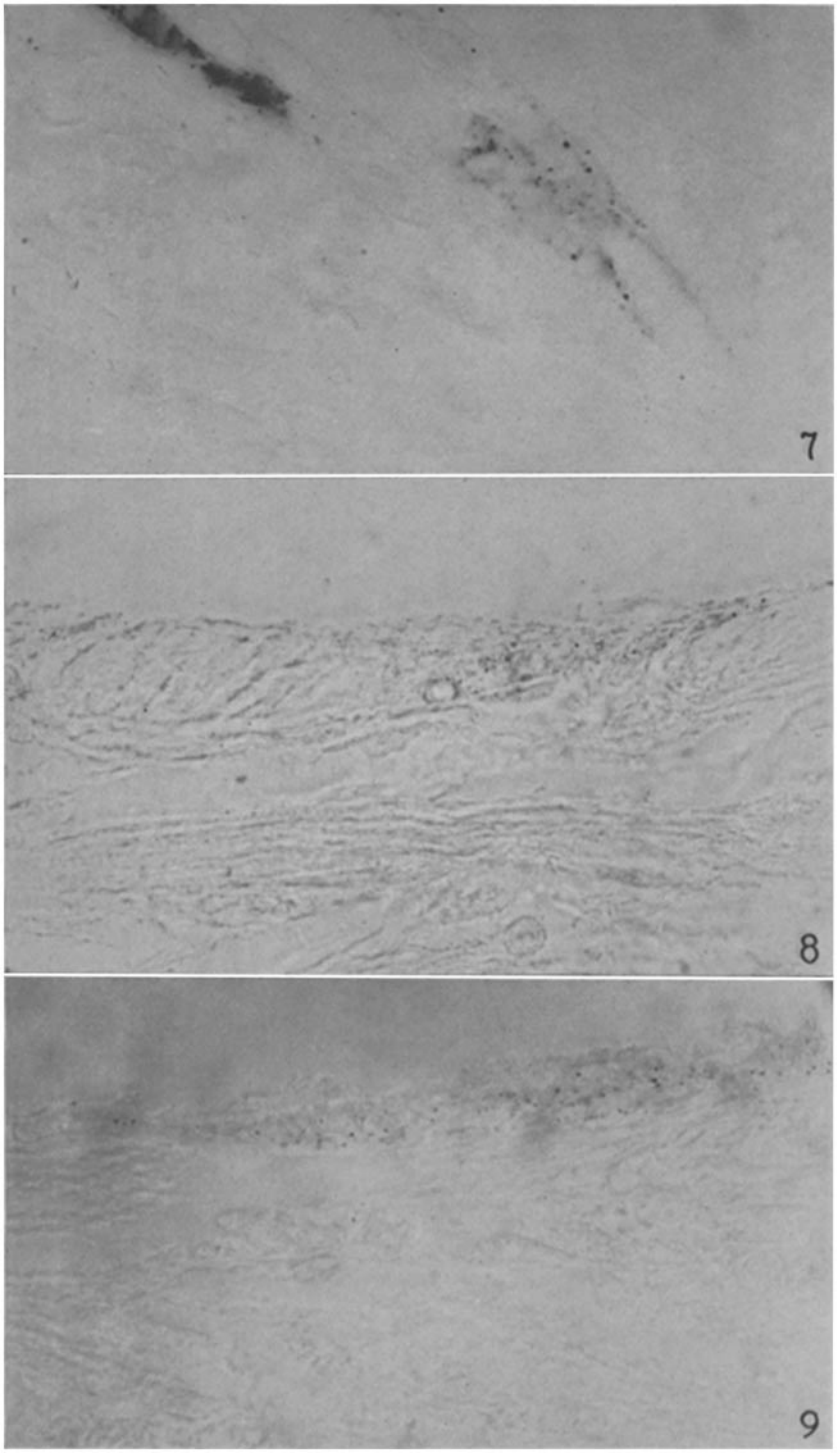
Photographed by Louis Schmidt

(Rake: Invasion of body through olfactory mucosa)



Photographed by Louis Schmidt

(Rake: Invasion of body through olfactory mucosa)



Photographed by Louis Schmidt

(Rake: Invasion of body through olfactory mucosa)