VITAL STAINING OF THE LEPTOMENINGES

By H. H. WOOLLARD,
Demonstrator of Anatomy in the University College, London

(*From the Anatomical Laboratory of the Johns Hopkins University*)

In 1913 Goldmann (6) published an account of his experiments with vital dyes injected into the subarachnoid space of rabbits and dogs. The subarachnoid space was reached by trepanation of the skull or by laminectomy of the vertebral column. In rabbits the dose used was 0·5 c.c. of a 0·5 per cent. solution of trypan blue, while in dogs, 2 to 2·5 c.c. of a 1 per cent. solution were given. The immediate reaction of the animals demonstrated an extreme toxicity of the injected substances, for muscular spasm, tonic and clonic contractions, acceleration of respiration and heart-beat, then coma, and death of the animal in about nine hours followed. The author drew attention to the heightened toxicity of foreign substances when introduced by way of the cerebrospinal fluid and mentioned various observers who had demonstrated the increased toxicity of such substances as morphia, cocaine, potassium ferrocyanide, methylene blue and ferrocyanates when injected in the subarachnoid space.

Taking the most favourable of these experiments (those in which the animals survived the longest period after the injection), Goldmann made the following observations: The surface of the spinal cord was stained to a depth of 2 mm. throughout its whole extent, and the dye could be followed out around the spinal ganglia. The cerebral hemispheres exhibited a coloration similar to that of the spinal cord, but the stain was of a lighter hue. The blue was especially deep at the base of the brain and passed outward along the optic nerves to the corneo-scleral junction and along the olfactory nerves to the nasal cavities. The convexity of the brain was uncoloured, and only along the larger vessels could blue stripes be seen. The stain invaded the ventricles of the brain and the central canal of the spinal cord. Outside the central nervous system the trypan blue was seen in the sheaths of the large blood-vessels and in the liver and kidney. These findings occurred without exception in animals which exhibited the toxic symptoms described above.

Microscopically, Goldmann found the leptomeninges to be stained a diffuse blue. The pial blood vessels entering the brain substance were coloured, as were the nuclei and network of the glial cells. The ganglion cells of the anterior and posterior horns always showed a deposit of stain in the nucleus and their processes were distinctly outlined by the dye. The glial and pyramidal cells of the cerebral cortex and the Purkinje cells of the cerebellum were customarily

coloured with blue. When weak doses were used the ganglion cells appeared free from dye, but even in these cases, when observed with the oil immersion lens, fine blue granules could be found in the cells of the anterior and posterior horns. This coloration was not diffuse and the nucleus was unstained; Goldmann therefore concluded that they were not moribund cells. The pial septa were everywhere coloured and the cells lining the perivascular spaces showed blue granules. The cells in the arachnoid and pia bearing the stain and those extending from these membranes into the brain he regarded as similar to the so-called "pyrrhol" cells of the liver and spleen and connective tissues.

Goldmann's experiments seemed, on critical analysis, to be complicated by the toxicity of the dye in the dosage used for subarachnoid injection. This intraspinous toxicity was indicated by the reactions of the animals to such administration of the dye-stuff and by the more or less diffuse staining of the cellular elements of the nervous tissue itself. The present report is based on a series of experiments, which though similar to those of Goldmann have been planned with the object of avoiding the obvious limitations of his earlier work, and they have afforded definite information regarding the cell-morphology in the subarachnoid space.

VITAL STAINING UNDER NORMAL CONDITIONS

The animals chosen for the work were cats; the route of injection into the subarachnoid space was by an occipito-atlantal puncture into the cisterna magna (cerebello-medullaris). The dye used was trypan blue and the strength found most satisfactory was a 0·1 per cent. solution. The dye was first made up in a 1 per cent. aqueous solution; this was diluted with Ringer's solution to the desired strength. The procedure followed consisted of etherization of the animal, sterilization of the chosen area and puncture into the subarachnoid space with a suitable lumbar puncture needle. After release of the cerebrospinal fluid, a syringe containing the selected amount of dye (6 to 8 c.c.) was attached to the needle and the solution slowly injected. When 6 c.c. had been injected, the animal frequently exhibited a tonic spasm, with extension of the limbs, arching of the back, and extension of the head. This phenomenon, apparently due to increase in intracranial tension, passed off in a few minutes, and after recovery from the ether anaesthetic, the cat behaved normally. The injections were repeated on alternate days; between the injections the animals ate well, cleansed themselves and were active and normal in every respect. The cerebrospinal fluid removed was examined on each occasion but with the experimental procedure carried out as outlined no cells were ever found in it. As was mentioned a 0·1 per cent. solution of the dye was found to be the most satisfactory, for with this dosage the cats were perfectly normal in the intervals. If a 0·2 per cent. solution was used the dose was fairly well borne, but between the doses the cats were lethargic. If a 0·3 per cent. solution was given, the cats, though they exhibited no muscular phenomena, were torpid, ate little and did not cleanse themselves.

The following protocols give a summary of the cats stained according to the above procedure:

Cat 1. This cat was given, in three injections, 15 c.c. of a 0·1 per cent. solution of trypan blue in Ringer's solution. The cerebrospinal fluid, removed before each injection, contained no cells. The cat remained in good condition and was killed 48 hours after the last injection of the dye and fixed in 10 per cent. formalin by aortic injection.

Cat 2. This cat received 22 c.c. of a 0·1 per cent. trypan blue solution. The initial dose given in this case was 10 c.c. After this injection the animal became lethargic. Two subsequent injections of 6 c.c. were given. As the animal was becoming weaker it was killed and injected as above.

Cat 3. This cat received, in seven injections, 42 c.c. of the 0·1 per cent. trypan blue solution. The cerebrospinal fluid remained free from cells. The cat was perfectly well during the intervals and was killed and fixed in 10 per cent. formalin 48 hours after the last injection.

Cat 4. This cat received, in eight injections of 8 c.c. each, 64 c.c. of the 0·1 per cent. trypan blue. The animal remained in excellent condition throughout the experiment and the cerebrospinal fluid was always free from cells. The animal was killed, fixed in 10 per cent. formalin by aortic injection eight hours after the last injection of trypan blue.

Cat 5. The dye received by this animal had salted out before it was injected and after a dose of 6 c.c., the animal exhibited convulsive movements. The spasms came on five minutes after the cessation of the injection; coma soon supervened and death followed quickly. Examination showed everywhere an indiscriminate staining of meninges and nerve cells indicating the well-established manner of staining of injured or dead tissues.

Macroscopic Examination. After fixation of the animal by injection through the heart with 10 per cent. formalin, the skull and vertebral column were detached from the rest of the body and the vertebral arches were opened; this block of tissue was placed in 10 per cent. formalin for three or four days for further fixation. At the end of this time the central nervous system with membranes intact was detached completely from the bone.

The whole surface of the central nervous system and membranes appeared intensely blue. The only unstained structures were the spinal ganglia and the pituitary gland. On closer examination of the spinal nerves, the anterior and posterior roots appeared as white strands clothed in blue membranes which ceased on the proximal side of the ganglia. The cranial nerves passed out through the cranial foramina as white strands. The Gasserian ganglia were perfectly white. The superficies of the optic nerve and chiasma were blue and the investing blue membrane reached the posterior pole of the eyeball. The olfactory bulb was enveloped in blue but the stain did not accompany the olfactory fila and no stain appeared in the olfactory mucous membrane. The intensity of stain was more marked in the area adjacent to the site of the injection. In those animals which were lightly stained the dye was most

marked over the cervical cord, base of the brain, cerebellum and occipital poles of the cerebrum. In those more heavily stained, the coloration attained a more uniform intensity, the convexity of the cerebral hemispheres, as well as the caudal end of the spinal cord, showing the same intensity as around the site of injection. When the dura was raised up it was pale and semi-transparent and quite free from the dye. The membranes underlying the dura were intensely blue. Transverse sections of the spinal cord showed a faint blue stain which appeared to be confined to the white matter of the cord. The blue colour faded out before the grey matter was reached and the latter appeared entirely unstained. Cross sections of the brain and cerebellum showed a faint blue coloration of the cortex extending inward from the pia. The ependymal lining of the ventricular system was free from the dye and the choroid plexuses were pale and unstained. The dura in the hypophysial region when lifted away disclosed the pale pituitary body embraced circumferentially around its attachment to the brain by the blue coloured arachnoid.

At the site of injection there was always a slight leakage of trypan blue into the adjacent extradural tissues. Lymphatics could be traced to the large vitally stained lymphatic glands at the base of the skull. These glands bore a considerable amount of dye but the dye had apparently reached them from the site of extravasation and not from their cranial connections. In the more lightly stained animals these glands were only slightly coloured. The liver, spleen and kidney, showed no signs of visible coloration in any of the animals.

These observations stand in marked contrast to the failure of the central nervous system to stain when the vital dye reaches it through the blood stream. For after intravascular injection in a healthy animal only the dura and the choroid plexuses are deeply stained, though the pituitary and the adjacent tuber cinereum are faintly coloured (Goldmann). Further, Wislocki and Putnam (16) found that the small areae postremae (areas rich in blood-vessels and glial tissue) of the fourth ventricle were coloured by the dye. None of these areas took up the stain from the subarachnoid space. Furthermore, the leptomeninges on vital staining through the blood stream are uncoloured, whereas on subarachnoid injection these membranes are intensely stained. The faint bluish coloration of the peripheral zones of the nervous tissues on subarachnoid injection of the vital dye is also to be contrasted with the lack of staining of these zones after intravascular injection of the trypan blue.

Microscopic Examination. With the aid of the binocular microscope the membranes were dissected off the cord and the brain. The dura mater from all regions of the brain and spinal cord was entirely free from any evidence of staining with trypan blue. The arachnoid was carefully raised from the pia and the numerous delicate trabeculae uniting the arachnoid to the pia were divided with finely pointed scissors. The membranes were counterstained in carmine and mounted as films, care being taken in the case of the arachnoid to have the subdural surface next to the slide. The blue colour of the arachnoid enabled one, in dissecting it away from the pia, to see that it did not enter any of the

fissures of the cord and that it embraced the anterior and dorsal nerve roots ceasing proximally to the spinal ganglia on the dorsal root and at an equivalent position on the ventral roots.

The histology of the arachnoid and pia has been described by Weed (14) as follows (p. 398):

"The arachnoid is a cellular structure with a delicate supporting reticulum of white fibrous and yellow elastic fibrils, covered on both sides by characteristic flat cells. From the inner side of this membrane project small, easily-broken trabeculae, upon which are contained the cells forming the inner lining of the arachnoidea. The core of such an arachnoid trabecula is composed of a few connective tissue fibrils, united on the outside with the fibrous framework of the membrane and on the inner side with the sub-pial tissues...."

"The pia mater,...closely invests the nervous system, continuing into the cerebral sulci and into the medial fissures of the cord. Actually...it is essentially constituted by the 'visceral' reflection of the cells of the arachnoidea, but it possesses in addition an underlying supporting structure of a few connective tissue fibrils and neuroglial elements."..."Quite similarly, all of these vessels perforating the fluid-spaces between pia and arachnoid are likewise covered by an inclosing investment of mesothelial cells. Thus, the sub-arachnoid space (or, better, spaces) become a fluid-bed, everywhere lined by the typical, very low mesothelial cell."

The essential structures mentioned in this description were adequately demonstrated in the spread preparations of the leptomeninges of these vitally stained animals, but other elements in the arachnoid should be mentioned. These are the arachnoid clusters or whorls of flattened cells which may be confined to the arachnoid or may hypertrophy and penetrate the dura, and secondly the arachnoid villi—projections of the arachnoid, covered by a layer of mesothelium—into the walls of the great sinuses. Both of these structures have been recently described by Weed (13, 15).

In the preparations of the leptomeninges three orders of cells taking up the dye could be distinguished in the arachnoid and pia. The first was the clasmatocyte. In the cytoplasm of these cells the dye was accumulated in granules of various size and depth of colour. The dye had the appearance of being stored in vacuoles ("segregation vacuoles," Shipley (12)). Other features, such as the well-defined nuclear membrane, the scanty chromatin, the eccentric position of the nucleus, the presence of a concavity in the nucleus towards the main protoplasmic mass were all present. The shape and size of the cells varied somewhat but they were usually round or oval and slightly larger than a polymorphonuclear leucocyte.

The second cell that could be distinguished by the presence of granules of trypan blue in its cytoplasm was the mesothelial lining cell. These could be seen to best advantage on the trabeculae. Elongated, flattened out, with rod-like nucleus and scanty cytoplasm, they were characterized by the presence of a few granules of the dye. These granules were very tiny and discrete.

They were sparse in number and close together in the cytoplasm, near to either pole of the nucleus. The dye was confined to these two areas and did not extend into the remainder of the cell.

When the films of animals which had been more heavily stained were studied, the type of aggregation of the dye-granules within these mesothelial cells was noted to be somewhat different from that in the animals which had received smaller amounts of stain. Furthermore, in these animals cell multiplication had taken place. Though mitotic figures were more easily found in the cells on the trabeculae, yet the same process could be made out in the flattened areas between the origin of the trabeculae. Secondly, many of the mesothelial cells appeared to be rounding up and acquiring a cuboidal form, with the round, palely staining nucleus occupying the centre of an increased amount of cytoplasm which was studded with fine granules of blue. In presumably older cells, which were still lying spread on the trabeculae or so far rounded up that their attachment to the trabeculae had become quite attenuated, the nucleus had become eccentric and was concave toward the part of the cell where the cytoplasm had accumulated. In this intracellular area the trypan blue was not accumulated and the granules were coarser though not so large as those contained in the clasmatocytes. Further small vacuoles were distinguishable.

Thus under the influence of the irritation of the dye there had been induced in the mesothelial cell a series of changes of which the final result was to produce a mononuclear phagocytic cell of a morphology somewhat similar to that of the clasmatocyte, yet distinguishable by the smaller amount of the dye by less coarse clumping of the blue granules, and by the fewer and rarer vacuoles.

The third cell containing granules was the fibroblast. A pale round nucleus with a narrow ring of cytoplasm and many branching processes characterized this cell which took up the dye in a notable way. Around the nucleus in the ring of protoplasm a perinuclear ring of fine discrete granules of dye could be seen and the same type of fine discrete granules was noted in the processes of the cells. No perceptible changes in the form of the fibroblast were encountered on comparing the films from lightly or more heavily stained animals. Fibroblasts were found to be relatively few in the leptomeninges.

In the villi and arachnoidal cell-clusters occasional mononuclear phagocytic cells containing aggregations of dye were seen but the main mass of cells making up these structures was free from trypan blue. In a cell-cluster of the arachnoid which had hypertrophied and advanced towards the dura an occasional dye-bearing cell beyond the level of the dural surface of the arachnoid was visible. The dura never contained any dye-bearing cells and the presence of vital dye in these cell clusters bears out the arachnoidal origin of these dural cell-inclusions.

In the pia the same analysis of the cells held good. The endothelium of the blood-vessels contained none of the dye. The dye accompanied the pial tissue for a short distance into the choroid plexuses but none reached the choroidal

epithelial cells. In the region of the pituitary, the dye was present in the arachnoid on the anterior surface of the gland. Though a continuation of the subarachnoid space (Hughson (5)) has been shown, by the injection of potassium ferrocyanide and iron ammonium citrate under certain experimental conditions, to encircle the pituitary, yet with trypan blue, the dye was not found about the base of the gland.

As is fairly well established, the perivascular spaces which surround the entering vessels of the spinal cord and brain are lined by mesothelial cells of the pia which continue inward for a certain distance. In the spinal cord of the specimens of the present series, flattened mesothelial cells bearing fine discrete granules of dye in the cytoplasm at either nuclear pole could be traced inward along the entering vessels. In some instances they could be followed across the white matter and still in continuity with the perivascular space could be found in the grey matter of the cord. In the cortex a similar phenomenon was visible. Along an entering vessel these mesothelial cells bearing granules of dye could be traced and could be found for a short distance along the lateral branches of the vessels, if these were comparatively large. However, they were not found to line the whole space, for the cells with blue granules were limited to the more peripheral portions of the perivascular channels; in general, however, they continued to the smaller arterioles and venules before ceasing to line the perivascular cuff. In the perineuronal space and about the capillaries there were no dye-bearing cells and here the lining cells appeared to be neuroglial.

The epidermal cells—nerve cells, ependymal cells, and neuroglial cells—in no instance contained the vital dye when administered by the subarachnoid route. Blocks of nerve tissue were sectioned in celloidin and carefully examined but the nerve cells were never found to contain dye. The ventricular lining, the cells of the central canal, and the medullary vela were free from stain. Under pathological conditions (MacCurdy and Evans (9), infantile palsy; Macklin (7), MacCurdy (10), Mertzbacher (11), Essick (4), with experimental inflammation), vital staining of nerve cells and neuroglia occurs after intravascular injection, but under these conditions the cells are dead or damaged.

Thus it seems logical to conclude that when a vital dye, such as trypan blue, is injected into subarachnoid space it remains confined to the membranes which bound that space: the dye is taken up in great quantities by the mesothelial lining cells, by the clasmatocytes, and to a slight extent by the fibroblasts. The presence of dye-bearing cells determines accurately the limits of the mesodermal leptomeninges and thus it becomes possible to establish the pial boundaries of the perivascular spaces of the central nervous system and to substantiate the arachnoidal origin of the cell-inclusions in the dura.

VITAL STAINING UNDER CONDITIONS OF IRRITATION

It was believed that if such a vitally stained animal were subjected to further irritation of the leptomeninges so that free cells occurred in the cerebro-

spinal fluid, some light might be shed on the question as to what cells contributed to the formation of these free cells. The reaction of the mesothelial cell to the irritation of the dye and the results obtained by Essick (3) when the cells were irritated, furnished very strong presumptive evidence that, besides the group of mononuclear phagocytic cells derived from the clasmatocytes, additional free cells might be derived from the mesothelial elements. The mesothelial cell, under slight irritation, stored the dye in a rather specific fashion, but with the stimulus of greater amounts of dye, a tendency to vacuolation and to storage of the dye in larger particles was apparent. Therefore, the storage of dye granules afforded no definitive criterion for the establishment of the origin of a particular mononuclear phagocytic cell, since the leptomeningeal mesothelial cell, while still sessile, became transformed into a mononuclear phagocytic cell not sharply separated from the clasmatocyte type. The definite differences which still persist have been recorded in foregoing paragraphs, and they serve to furnish a criterion for distinguished cell-types in the fluid. The recent work of Cunningham (2) on the serosal cells in peritoneal exudates gave an added interest to this investigation for he was able to follow these cells through degenerating stages to death. Therefore, to enquire into the further fate of the mesothelial cell of the leptomeninges, a series of experiments was carried out, and the reactions of these cells when stained vitally and stimulated by the presence of non-toxic particulate matter were observed.

Following the technique described by Essick (3), cats which had been stained with trypan blue by the atlanto-occipital route were injected subsequently with fragmented blood corpuscles into the subarachnoid space. The injection consisted of 5 c.c. of blood corpuscles, laked in distilled water and rendered isotonic by the addition of five times normal concentration of sodium chloride, potassium chloride, and calcium chloride. The cerebrospinal fluid was drawn off after the injection at intervals corresponding to 18 hours, 24 hours, and 40 hours. Films of the fluid were made and examined in the warm box; others were stained with Wright's stain and carmine.

The following condensed protocols indicate the exact technical procedure:

Cat 1. Subarachnoid injection of 34 c.c. of 0·1 per cent. trypan blue.
„ „ 5 c.c of laked blood 48 hours after injection of trypan blue.

Cat 2. Subarachnoid injection of 16 c.c. of 0·1 per cent. trypan blue.
„ „ 16 c.c. „ 0·2 „ „
„ „ 8 c.c. „ 0·3 „ „
5 c.c. of laked corpuscles 24 hours after last injection of the blue.

Cat 3. Subarachnoid injection of 16 c.c. of 0·1 per cent. trypan blue.
„ „ 16 c.c. „ 0·3 „ „
„ „ 8 c.c. „ 0·3 „ „
5 c.c. of laked blood 24 hours after last injection of trypan blue.
5 c.c. „ „ 48 „ first „ blood.

Films of the leptomeninges prepared from the first of these animals showed, that, as Essick had found, the initial reaction to the laked corpuscles was the pouring into the cerebrospinal fluid of a great number of polymorphonuclear leucocytes. At 18 hours after the injection the polymorphonuclears were greatly in excess, mononuclear phagocytic cells being relatively few. (The film showed numerous red corpuscles derived from the injection.) The films made 24 hours after injection showed a great increase in the number of mononuclear phagocytic cells. Many polymorphonuclears and injected corpuscles were still present. Some films were made on slides that had been prepared with a layer of neutral red according to the method used by Dr Sabin (verbal communication); two kinds of mononuclear phagocytic cells were distinguished. The larger kind seemed to be a typical clasmatocyte with irregular cell outline, with granular protoplasm, vacuoles, and containing neutral red granules and fragmented or entire red corpuscles. The other mononuclear phagocytic cells were smaller in size, more round in outline; the granules of neutral red taken up were much finer and vacuoles were not obvious. This differentiation of the mononuclear cells into two types was not apparent in films prepared from this animal 40 hours after the injection of laked blood. While the polymorphonuclear cells were few in number, the mononuclear elements were also very scarce and were all of the clasmatocyte type.

The animal was then killed and fixed by injection of 10 per cent. formalin through the heart. The specimen of the nervous system showed relatively slight coloration with the blue. Spread preparations of the arachnoid and pia were stained and mounted as before. The same cellular pictures described for the vital dye films were again identified. Many polymorphonuclears were seen on the spreads and red corpuscles, still uningested, were present. The trabeculae showed most clearly the changes which had occurred. Cell division was abundant. Cells rounding up, becoming plumper and disengaging themselves from the trabeculae could be seen. These cells before disengaging themselves seemed to have undergone division because wherever a cell seemed to be nearly free a small cell with its nucleus opposite that of the pedunculated cell was apparent. In this way the trabeculae were not denuded of cells. These disengaging cells had concentrated their vital dye towards the major protoplasmic mass and the nucleus had become eccentric. Fragments of red corpuscles and vacuoles lined with blue were apparent in them.

These findings confirm the results of Essick. The use of trypan blue though not of determining value in this case as a criterion of cell-type among the free mononuclear cells of the cerebrospinal fluid did afford preparations which suggested strongly that the mesothelial cell as it became transformed into a free mononuclear phagocytic cell resembled in certain particulars the clasmatocyte, two essential points of difference remaining. The mononuclear phagocytic cell derived from mesothelial cells of the leptomeninges was smaller and the granules of dye, whether neutral red or trypan blue, were finer than in the true clasmatocyte. The maintenance of these differences permits the assump-

tion that the cells are genetically separate and that they do not, under the conditions of observations, become identical.

Cats 2 and 3 were heavily stained and the films of cerebrospinal fluid displayed polymorphonuclear leucocytes and mononuclear phagocytic cells of the clasmatocyte type. Study of warm box preparations and stained films did not enable one to distinguish varieties in the mononuclear phagocytic cells. A few mononuclear cells without any trypan blue granules were encountered—perhaps desquamated mesothelial cells. The clasmatocytes free in the cerebrospinal fluid were heavily stained with trypan blue. On examining the membranes there could be no doubt that lining cells were rounding up and engulfing red blood corpuscles and polymorphonuclears; the dye was in fairly large granular masses and vacuoles were present. Some of these cells were quite round; others, spindle shaped, with their poles prolonged into fine processes; others were slender and elongated. They were superficial to the clasmatocytes, which could be identified at a deeper level in the spread-preparation.

It was hardly possible to distinguish varieties in the mononuclear phagocytic cells in the cerebrospinal fluid and almost equally difficult in the films of the fluid, but here in the spread-preparations the difference in position assured one of the separate identity of lining cells and clasmatocytes. Mesothelial lining cells whose transformation to mononuclear phagocytic cells was complete lay in continuity with less advanced cells and formed the surface of the preparation. Beneath this layer true clasmatocytes could be brought into focus.

DISCUSSION

Mallory (8) has suggested that the mesothelial lining cell of the subarachnoid space is a flattened out fibroblast. The fibroblast in these spread preparations behaved in the characteristic fashion toward trypan blue, determined by many investigators; no evidence was found of this highly differentiated cell becoming a free mononuclear phagocytic cell in the cerebrospinal fluid. The mesothelial covering cell of the leptomeninges presented a reaction to trypan blue which was characteristic. First a few fine granules appeared in the cytoplasm at either pole of the nucleus. Then as the dye, on further injections, increased in amount, these cells underwent division, rounded up and showed many more fine granules of dye in their cytoplasm and in the most extreme case (Cat 5), many cells still lying on the trabeculae showed an eccentric nucleus, and accumulation of dye in the major portion of the cytoplasm, and vacuoles. Furthermore, they ingested laked blood corpuscles, fragments or whole polymorphonuclears and became in certain respects indistinguishable from a clasmatocyte. Whether they became free in the cerebrospinal fluid or not, these experiments have not conclusively determined.

In a study of the serosal cells of the peritoneum, Cunningham found that these cells did not contribute to the mononuclear phagocytic cells of peritoneal exudates. His review of the literature shows how difficult it is to interpret the

evidence and this study necessarily suffers from the same defect. It appears, however, that the mesothelial cell of the subarachnoid space is not identical with the serosal cell of the serous-cavities but is a less differentiated cell, somewhat more primitive in type, capable of becoming under sufficient stimulation a mononuclear phagocytic cell.

Other studies on the inflammation of the brain and subarachnoid space (Macklin (7), MacCurdy (10), Ayer (1), Essick (4), and Mertzbacher (11)) have given the mesodermal elements of the pia and arachnoid as the chief source of the mononuclear phagocytic cells. Mertzbacher indeed included the neuroglia, the lymphocytes of the blood and the endothelium of capillaries amongst the sources of his "Abraumzellen." Yet an analysis of the above shows the elements of the pia-arachnoid as the chief source. Our studies indicate that the mesodermal elements of the pia and arachnoid capable of becoming mononuclear phagocytic cells, are not only the clasmatocytes but also the mesothelial lining cells; though we did not succeed in obtaining conclusive demonstration of the presence of the second group free in the cerebrospinal fluid, yet the finding of two clearly defined types of mononuclear phagocytic cells in the preparations and films of the fluid, in addition to the evidence of reaction of these cells while still attached to the membrane, leads us to believe that the lining cells of the subarachnoid space form such free mononuclear phagocytic cells in the cerebrospinal fluid.

SUMMARY

1. The injection of vital dye into the subarachnoid space outlines the confines of that space, demonstrating the mesothelial lining cell completely enclosing the fluid-channels and continuing inward in the perivascular channels.

2. The dye is ingested by the lining mesothelial cells, by clasmatocytes, and by fibroblasts.

3. Under the influence of the dye and of partially destroyed red blood corpuscles, the lining cells become transformed into vacuolated, mononuclear, phagocytic cells.

4. The free mononuclear phagocytic cells of the cerebrospinal fluid under these conditions are derived from clasmatocytes and also in all probability from the mesothelial lining cells.

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