THE FACTORS WHICH GOVERN THE PENETRATION OF ARSENIC (SALVARSAN) AND ANILINE DYES INTO THE BRAIN AND THEIR BEARING UPON THE TREATMENT OF CEREBRAL SYPHILIS.[1]

By JAMES McINTOSH, M.D.,
Late Research Scholar of the Grocers' Company, &c.,

AND

PAUL FILDES, M.B.,
Assistant Bacteriologist to the London Hospital.

(*From the Bacteriological Laboratory of the London Hospital, Professor William Bulloch, F.R.S.*)

In a previous paper [2] we have shown that after intravenous injections of salvarsan and neosalvarsan no arsenic can be found in the brain substance, this result being due not to any lack of affinity between the brain-cells and the drug, but to an obstruction to the passage of the drug from the blood-stream into the brain substance.

In the present paper we detail experiments which we have devised to throw light upon the factors involved in penetration or lack of penetration into the brain. We required for this investigation a group of substances which were relatively non-toxic when injected intravenously and readily recognizable in the tissues after the death of the animal. Various dyes, some of which had already been investigated by Ehrlich, were selected for our purpose.

Our mode of procedure was as follows :—

A rabbit was injected intravenously with a large dose of an aniline dye and killed about five minutes later by air embolism—10 c.c. of air being injected into the ear vein. The animal was then dissected and the distribution of the stain noted in the tissues generally. The brain and cord were next exposed and examined. The cerebrospinal fluid was collected in a capillary pipette by puncture of the membranes after exposure of the cord; by this method the fluid may be obtained

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macroscopically free from blood. The pipettes were viewed along their long axis in order that small traces of stains might be detected.

By the method of intravenous injection stains may be divided into two groups, namely, those which stain the central nervous system and those which do not.

Group I.—STAINS WHICH COLOUR THE TISSUES GENERALLY AND ALSO THE CENTRAL NERVOUS SYSTEM.

(1) *Methylene Blue.*

This stain was used as an intravital stain by Ehrlich. It is very rapidly reduced to a colourless compound by the tissues, and this fact must be borne in mind when investigating its distribution within the body.

The effect of methylene blue is as follows :—

Experiment 1.—A rabbit (1,300 grm.) was injected intravenously with 1·5 c.c. of a saturated solution of methylene blue ("medicinal pure" Grübler). The animal immediately became restless and tinged with blue. This coloration rapidly passed off and the symptoms were disappearing when the rabbit was killed by air embolism five minutes after the injection. The internal organs were examined immediately and found to be uncoloured, but the muscles were slightly blue and this became intensified on exposure to air.

The membranes of the brain and cord were uncoloured, the cerebro-spinal fluid was also uncoloured and remained so even after the addition of hydrogen peroxide. The brain was at first colourless, but on exposure to air became distinctly blue ; the cord on the other hand was uncoloured and remained so.

The brain and cord were placed in Bethe's solution [2] for the fixation of methylene blue. The blue colour then became more intense and was seen to be confined to the grey matter of both structures. After fixation and embedding in paraffin sections were cut and the blue staining was found to be diffuse. No intracellular granules were observed and no differential staining of the tissues.

Experiment 2.—In a second experiment a rabbit (2,000 grm.) was injected with a larger dose, 10 c.c., of a saturated solution. Toxic symptoms were more profound, but were passing off when the animal was killed five minutes later.

[2] Dissolve ammonium molybdate (1 grm.) in 10 c.c. of distilled water and add 1 c.c. of hydrogen peroxide—the solution will become yellow—then add one drop of hydrochloric acid.

The appearance of the tissues was similar, although in this case the lungs were distinctly stained.

Thus, after the injection of methylene blue the distribution of the stain in the central nervous system is as follows: Meninges, unstained; cerebrospinal fluid, unstained; grey matter, stained diffusely, no granules; white matter, unstained.

(2) *Neutral Red.*

This stain was also used by Ehrlich for intravital purposes. It has very little toxicity, and is rapidly excreted by the kidneys.

Experiment 3.—A rabbit (1,500 grm.) was injected intravenously with 15 c.c. of a saturated solution of neutral red (Grübler, "for intravital injection"); tetanic spasms came on before the injection was finished and rapidly increased until complete rigidity was present. These symptoms, however, soon began to pass off and the animal was killed ten minutes later. General staining was present externally.

On dissection the organs were found to be stained, the liver and kidneys being dark purple and the muscles bright red.

The meninges of the central nervous system were slightly pink, but the cerebrospinal fluid was entirely colourless. The brain, on the other hand, was intensely stained a dark red, while the cord was pink. Incisions into the brain and cord showed that the colour was confined to the grey matter. Portions were fixed in formalin and embedded in paraffin. In sections the coloration of the grey matter was found to be diffuse, although there appeared to be a slight differential staining of the cortical layer of the ganglion cells. No granules, however, were observed in these cells.

Experiment 4.—In a further experiment a rabbit (1,600 grm.) was injected with 7 c.c. of the saturated solution and an exactly similar distribution of the stain was found. In this case, however, a slight coloration was present in the cerebrospinal fluid due to a small admixture of blood.

Amongst the other stains which affected the grey matter of the central nervous system were alizarin blue and malachite green; while indophenol and aurantia did so only slightly. In no case, however, was the effect quite so marked as with neutral red and methylene blue, perhaps because the less solubility and greater toxicity did not allow of their use in such large doses.

Group II.—STAINS WHICH COLOUR THE TISSUES GENERALLY, BUT NOT THE CENTRAL NERVOUS SYSTEM.

(1) *Fluorescine.*

Experiment 5.—A rabbit (1,500 grm.) was injected intravenously with 5 c.c. of an alkaline watery solution of fluorescine (1 per cent.). No toxic symptoms followed and the animal, distinctly yellow, was killed after five minutes by air embolism.

On examination the internal organs were slightly yellow, while the kidneys were intensely stained. The muscles were golden yellow.

On exposing the central nervous system, the meninges were found to be stained but the cerebrospinal fluid was quite colourless. Both the brain and the spinal cord were entirely free from colour.

Experiment 6.—Another rabbit (1,500 grm.) was injected with 10 c.c. of the same solution of fluorescine. Exactly similar results were obtained as in the last experiment, that is, the brain and cord were unstained while the meninges were stained. In the cerebrospinal fluid a trace of fluorescine was detected, due, no doubt, to the presence of a slight quantity of blood which could be detected by the microscope.

(2) *Indigo-carmine.*

Experiment 7.—A rabbit (1,800 grm.) was injected with 20 c.c. of a saturated solution of indigo-carmine in saline solution. No toxic symptoms appeared and the animal became tinged green. In five minutes it was killed.

The internal organs and muscles were stained green. The meninges of the brain and cord were distinctly coloured, but the cerebrospinal fluid and the nerve tissue were entirely unstained.

Other stains which did not colour the central nervous system were found to be acid fuchsin, light green, trypan red, trypan blue, and pyrrhol blue.

From our experiments, a number of which have been given above, it is clear that the aniline dyes tested may be classified according as to whether, on intravenous injection, they stain the nervous tissues or not, and an examination of the physical properties of these substances might reveal the factors by virtue of which one group stains while the other does not.

From analogy with our experiments with salvarsan we argued that one stain did not colour the brain, while another did, because the former

did not penetrate. The blood capillaries of the brain are peculiar when compared with those of other parts of the body, in that they are surrounded by an extra adventitial sheath, and we supposed that this extra sheath might act as a barrier to certain drugs while permitting the passage of others. We therefore investigated the chemical formulæ of these stains, but were unable to find any atomic arrangement which was characteristic of the two groups.

Similarly the diffusibility of the stains did not correspond with their staining reactions.

The question of solubility appeared to be of more importance. Ehrlich [1] stated that neurotropic substances are lipotropic, and Overton [3] that before a substance could penetrate into a cell it must be soluble in the cell membrane, and these membranes being lipoids it follows that such a substance must be soluble in lipoid solvents such as alcohol and ether. The solubility of the dyes which we used in alcohol and ether did not correspond with their staining reactions, but when we used chloroform as a solvent we found that those stains which coloured the brain were soluble in chloroform, while those which did not were insoluble. It would appear, therefore, that the solubility in chloroform or related substances is an important factor in determining a passage from the blood into the nervous tissues or not.

On applying this test to salvarsan we found that neither salvarsan nor neosalvarsan were soluble in chloroform; we are, therefore, inclined to believe that this fact can account for their absence from the brain.

In order to test this suggestion we have examined a large number of organic and inorganic arsenical compounds, for the majority of which we have to thank Miss F. M. G. Micklethwait, Imperial College of Science. No very striking result was obtained, due no doubt to the fact that hardly any of these compounds were soluble in chloroform, and the few which were, were either completely insoluble in water or immediately hydrolysed by it. Certain combinations, however, of arsenic and aniline colour bases gave encouraging results, and after their administration arsenic was demonstrable in the brain, but owing to the War the work had necessarily to be suspended in this interesting stage.

Conclusions.

(1) Certain dye substances can pass directly from the blood to the brain substance proper without being found in the cerebrospinal fluid, while others fail to penetrate into the brain.

(2) The chief factor which governs the passage of the dyes is their solubility reactions.

(3) This is a peculiar solubility and not a general lipoid solubility.

(4) It corresponds to a solubility in chloroform and in water or perhaps to their partition coefficient in these liquids.

(5) The present-day arsenical remedies are, to some extent, inefficient in the treatment of syphilis of the central nervous system because they do not possess the necessary solubility to allow them to pass from the blood-vessels into the brain substance. Their relative inefficiency has nothing to do with their absence from the cerebrospinal fluid.

LITERATURE.

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