METACHROMATIC BODIES IN THE BRAIN

BY

MARION C. SMITH*

*From the Neurological Research Unit of the Medical Research Council,
National Hospital, Queen Square, London*

The purpose of this paper is to report the results of a study of the mucin, or mucin-like metachromatic substance, sometimes found in the white matter of the brain. This substance, occurring in small masses, has been referred to as "mucocytes." During the course of work on "grass sickness" in horses, these masses have been found; it therefore has become urgent, before continuing this investigation, to determine whether these metachromatic masses or bodies are mere artefacts; and if they are not artefacts, whether they are pathological or normal structures, and what their origin and nature may be. The term "mucocytes" is that most commonly used, but it may be misleadingly derivative, and will not be used here. Varying descriptions of this mucin-like substance have been given, and different views of its origin and significance expressed. It has generally been reported as occurring in the form of multilobulated grape-like areas throughout the white matter. Metachromatic bodies have been described by different workers as occurring in normal brains and in a large variety of pathological material, including senile dementia, epidemic encephalitis, Wilson's disease, paralysis agitans, and Schilder's disease. Different views as to the origin and significance have been expressed. The views on their origin which have been put forward fall roughly into two groups: first, it has been suggested that the metachromatic bodies are pathological, either of cellular origin or of extra-cellular origin; secondly, that the metachromatic bodies are artefacts.

The literature contains references to the works of many authors who hold the opinion that metachromatic bodies are pathological and arise from cells. Grynfeldt (1923) found microscopical multilobate patches in the white matter of the central nervous system of patients with senile dementia, paralysis agitans, and Wilson's disease. Working in association with Pélissier and Pagès (1924), he also found these patches in dogs poisoned with

formic acid. Pélissier (1924) studied similar areas in a case of "Wilson's disease following epidemic encephalitis." These areas gave the characteristic histo-chemical reactions of mucin. They were considered to be derived from glial cells. For these reasons Grynfeldt named them "mucocytes." Bailey and Schaltenbrand (1927) thought that the "mucocytic degeneration of Grynfeldt," and "acute swelling of the oligodendroglia" described by Penfield and Cone (1926) were identical. They studied a case of Schilder's disease, described the "mucocytic" degeneration present, and considered it an example of Grynfeldt's degeneration. Grinker and Stevens (1929) examined the brains in a laboratory collection from "cases of uræmia, arterio-sclerosis, encephalomalacia, toxic and epidemic encephalitis, Schilder's disease, and a variety of other conditions." They also examined so-called "normal brains" from hospital cases where the disease process was non-cerebral. They found that in most of the brains examined both acute swelling of the oligodendroglia and "mucocytic" areas were present. They thought that the mucocytic areas were the result of degeneration of the oligodendroglia; they believed that these cells, as they degenerate, swell and become mucoid, eventually bursting, and that they leave, as relics of themselves, coalescent masses of mucoid material.

Other authors have presented evidence in favour of their extra-cellular origin. Buscaino (1922), examining brains of patients with dementia præcox, and of animals poisoned with histamine, found free mucin in "grape-like areas of disintegration." He believed the masses were caused by abnormal amines circulating in the blood. He questioned the mucoid nature of the material, and named it "Substance X." He considered that it was derived either from degeneration of the myelin sheaths, or, less likely, from degeneration of ganglion cells. Lhermitte and others (1924) described mucin-like bodies in the brain of a patient with chronic epidemic encephalitis of Parkinsonian type, who died of

* Work undertaken on behalf of the Medical Research Council.

pulmonary tuberculosis. These bodies were free in the meshes of the white matter, showed no cell structure, and were spheroidal with irregular outlines. Their size was 20 to 30 μ : the bodies stained with basic and not with acid dyes; they gave the staining reaction of mucin, and were therefore thought to be mucin. Ferraro (1926, 1928) confirmed the metachromatic staining properties of the "mucocytes," and their solubility in chloroform, ether, pyridine, and partial solubility in acetone, absolute alcohol, and xylol. He found metachromatic concretions free in alcohol in which brains had been fixed; he considered "mucocytes" and these concretions to be identical. He also stated that metachromatic bodies could always be found in frozen sections treated with alcohol. He concluded that the grape-like areas of disintegration of "mucocytes" in embedded tissue, and the structures in frozen sections were identical. He claimed that the "mucocytes" were derived from myelin sheaths, and that any involvement of the oligodendroglia was evidence of phagocytosis in these cells. He also observed that "mucocytes" were never obvious in formaldehyde-fixed sections unless alcohol had been allowed to act on them. Alcohol may, in Ferraro's opinion, act as a sensitizer for "otherwise undemonstrable lesions" consisting of "disintegrating lipid or protein components of the myelin sheath." Thus, he considers that "mucocytes" are not necessarily artefacts. Their presence in normal brains does not invalidate this argument. It is possible that there are areas in a normal brain where the metabolism is to some degree altered without interfering with normal function: this alteration might lead to the presence of "mucocytes" in these areas. Unfortunately Ferraro did not specify the material he examined, and on which his conclusions were based.

The work briefly summarized above has favoured the biological origin of "mucocytes." Other investigators have brought forward evidence in favour of their being artefacts. Bielschowsky (1927) described areas of disintegration in the brain of a normal man free from pathological manifestations, executed at the age of 35. These areas were found in the centrum semi-ovale, corpus callosum, and areas where the grey and white matter are mingled. They were characterized by an absence of nuclei and by metachromatic staining properties. Bielschowsky thought that these mucous patches were derived from oligodendroglia by flowing out from these cells. The fact that there were fewer nuclei than normal was considered to be due partly to the degeneration of glial nuclei, and partly to an enormous increase in the volume of extra-vascular fluid. To what extent this effect was due to fixation was discussed

but not decided. Other workers such as Salustri (1924) and Ansalone (1924) considered "mucocytes" to be artefacts. The reasons for their opinions were: first, the fact that the areas have been described in the brains of apparently healthy men and animals as well as in pathological material; secondly, the fact that alcohol must be used to display them in frozen tissues.

It is impossible to know whether the appearances described in these papers refer to the same phenomenon; illustrations of Pélissier's findings differ widely from those of Lhermitte or Grinker and Stevens. The details of histological technique are not always mentioned: the duration of time between the death of the subject and fixation of the tissues, the fixative used, and the embedding method, are all points frequently omitted. As will be shown below, the technique used has a bearing upon the histological appearances of the masses. On the whole the appearances of the masses, as seen in frozen and celloidin sections, are similar to the illustrations published by Grinker and Stevens; while the same material prepared as paraffin wax sections resembles those of Grynfeltt and Pélissier.

Material and Findings

Metachromatic bodies were found in the brain of a man with Huntington's chorea, aged 57, and in two brains of young adult horses dead from grass sickness.* No pathological change was visible to the naked eye, either at the time of autopsy or when the brains were dissected after initial hardening. Throughout the investigation of these three brains, "normal" brains, both human and animal, were studied for comparison. These included: fifteen human brains of cases with non-cerebral lesions; brains from two normal horses, two normal sheep, and eight normal rats. A large number of slides of many neurological conditions from Dr. J. G. Greenfield's collection were examined; and various other histological material from the nervous systems of laboratory animals acquired during the course of other work was re-examined. The material was subjected to various procedures described below in order to elucidate the nature of these metachromatic bodies. The technical procedures are considered under the following six headings: (1) preparation of sections by different methods of embedding and application of a number of specific and non-specific stains; (2) examination of alcohol as a sensitizer for the production of metachromatic bodies; (3) examination of the reactions of metachromatic bodies to several solvents; (4) examination of the

* Grass sickness is a disease of horses of unknown aetiology characterized by marked apathy and somnolence and by intestinal stasis; it is commonly short, lasting twenty-four hours to three days, and frequently fatal.

effect of lipase digestion on metachromatic bodies ; (5) examination of factors, occurring between death and the preparation of sections, in the production of metachromatic bodies ; (6) examination of other possible factors.

EFFECT OF EMBEDDING AND STAINING METHODS

Methods.—After fixation and hardening of the three affected brains in 10 per cent. formol saline, representative blocks from throughout the brain were embedded in celloidin and paraffin wax. Adjacent areas were also examined by the frozen section technique. Similar areas of "normal" human and horse brains were identically treated. The celloidin sections of brains exhibiting metachromatic bodies were stained with: Ehrlich's hæmatoxylin and eosin, Anderson's iron hæmatoxylin and van Gieson's stain, Mayer's mucicarmine, Best's carmine stain, Bennhold's congo red for amyloid, azan, Foot-Masson's trichrome stain (Foot, 1933), Gros' stain for axis cylinders, Loyez' stain for myelin, Kultchitsky-Pal modification of Weigert's method (Anderson, 1929), and thionin or toluidine blue stain. The method of staining with thionin or toluidine blue was as follows. Sections were cut at 10 to 12 μ and transferred to 1 per cent. HCl in 70 per cent. alcohol for five minutes. They were thoroughly washed in several changes of distilled water for ten minutes. They were stained for twenty minutes in 0.04 to 0.02 per cent. Grubler's thionin or in 1 per cent. Grubler's toluidine in distilled water at 50° C. and differentiated in 95 per cent. alcohol with or without 5 per cent. cajuput oil. After clearing in xylol they were mounted in Mersol (a proprietary immersion oil produced by Flatters and Garnett).

Celloidin sections of "normal" brains were stained with: Ehrlich's hæmatoxylin and eosin, Anderson's iron hæmatoxylin, and van Gieson's stain, Mallory's phosphotungstic acid hæmatoxylin, Foot-Masson's trichrome stain, Gros' stain, Loyez' stain, and thionin.

Paraffin sections of brains exhibiting metachromatic bodies and "normal" brains were stained with: Ehrlich's hæmatoxylin and eosin, thionin, and Mayer's mucicarmine stain.

Frozen sections from brains exhibiting metachromatic bodies were stained with: toluidine blue, Scharlach R (Herxheimer method) (Anderson, 1929), Cajal's stain for astrocytes, Hortega's stain for microglia, Bielschowsky's stain, Victoria blue, and Mayer's mucicarmine method; while sections from normal brains were stained with: toluidine blue, Scharlach R, and Mayer's mucicarmine stain.

Findings.—In celloidin sections from brains containing metachromatic bodies, these bodies were seen as lobulated masses measuring on an average 100 μ ; they were most vividly stained with thionin, the colour varying from pink to grey-brown; with Ehrlich's hæmatoxylin and eosin, they stained a definite bluish purple. With Mayer's mucicarmine the masses were reddish purple, with hæmatoxylin alone there was no reddish coloration of the metachromatic bodies. Using Foot-Masson's and

Azan stains they were colourless or pale pink, and with phosphotungstic acid hæmatoxylin colourless. Loyez' and Gros' stains did not reveal these masses and sections so stained showed no abnormality. When thionin was used as a counter-stain on these sections the nerve fibres were seen in most instances to be apparently deflected to one side of the metachromatic bodies and occasionally a fibre passed through one. Kultchitsky-Pal stain showed no definite abnormality. None of the metachromatic bodies were bi-refringent, although small, bi-refringent particles were sometimes present between them (Table I and Figs. 1, 2, 3, and 4).

TABLE I
STAIN REACTIONS IN METACHROMATIC BODIES

Stain	Types of Section		
	Frozen	Paraffin	Celloidin
Thionin or toluidine	Pink	Red	Pink brown
Ehrlich's hæmatoxylin and eosin	Bluish	Blue-grey to colourless	Blue purple
Iron hæmatoxylin and van Gieson's stain			Blue purple or pale grey
Muci-carmine	Purple	Deep reddish purple	Reddish purple
Azan and Foot-Masson's stains			Pale pink to colourless

In the control "normal" human brains a very occasional pale structure of the shape of a "metachromatic body" was present but this was a rare finding. Such a structure stained a pale pink with thionin, bluish with hæmatoxylin and eosin. It did not stain with mucicarmine. In the "normal" horse brain structures as described above were a little more numerous and definite but were still very infrequent, in marked distinction to the very large numbers found in any microscopic field in affected horses.

When the paraffin sections of the brains containing metachromatic bodies were examined immediately after mounting, it was seen that some of the bodies were sufficiently large to be visible to the naked eye, measuring 300 to 500 μ in diameter. They were coloured a vivid red with thionin, reddish purple with Mayer's mucicarmine, and greyish with Ehrlich's hæmatoxylin and eosin. A short time after being mounted in Mersol or Canada balsam

(For legends see page 104.)

FIG. 1.—Metachromatic bodies in the white matter of the brain of a horse dying from grass sickness. (Celloidin section, $\times 200$.)

FIG. 3.—Metachromatic bodies in the white matter of the brain of a man with Huntington's chorea. (Celloidin section, $\times 350$.)

FIG. 5.—Metachromatic bodies in the white matter of the brain of a horse dying from grass sickness. (Paraffin section, $\times 200$.)

FIG. 2.—Metachromatic bodies in the white matter of the brain of a horse dying from grass sickness. (Celloidin section, $\times 400$.)

FIG. 4.—Metachromatic bodies in the white matter of the brain of a man with Huntington's chorea. (Celloidin section, $\times 600$.)

FIG. 6.—Metachromatic bodies in the white matter of the brain of a horse dying from grass sickness. (Paraffin section, $\times 650$.)

usually about thirty minutes, these masses had faded or dissolved, and irregular pale areas in the mesh-work of the white matter remained in their place. These areas were markedly larger than the masses seen in celloidin sections, and fewer in number, suggesting that smaller masses may have become diffuent and confluent. They were bi-refrangent both before and after staining; this bi-refrangement disappeared with the "fading" of the masses. No other bi-refrangent material was present in the sections (Table I, and Figs. 5 and 6). In the "normal" brains no appearances such as have been described above were found.

When frozen sections from the brains containing metachromatic bodies were stained with toluidine blue the bodies, similar to those seen in celloidin sections, were stained a definite pink colour. Scharlach R revealed no sudanophil material; no other abnormality was detected by use of the other stains. The metachromatic bodies were bi-refrangent; no other bi-refrangent material was present (Table I). In approximately half the "normal" brains examined, sections stained with toluidine revealed pinkish lobulated shreds along myelin tracts. A number of small pink bodies were also present. In the "normal" horse brain similar sections contained more definite pink multi-lobate masses. It has generally been accepted that such masses are derived from myelin. These bodies were not nearly so numerous as the metachromatic bodies in the affected brain; they tended to be smaller and more elongated, but otherwise in appearance they resembled the metachromatic bodies.

They did not stain with mucicarmine; they were mostly bi-refrangent (Table II).

SENSITIZATION OF METACHROMATIC BODIES BY ALCOHOL

Method.—Frozen sections were stained with toluidine blue, in the following manners: directly, omitting any form of alcohol before staining, after fifteen minutes in absolute alcohol, after twenty-four hours in absolute alcohol, and lastly after forty-eight hours in absolute alcohol.

Findings.—In brains exhibiting metachromatic bodies in which the sections were stained before treating with alcohol some pale indefinite pink patches were seen in the white matter. If the sections were stained after being in alcohol for fifteen minutes, these areas were far more clearly seen and stained more positively, as is characteristic of the metachromatic bodies. When the sections were in alcohol twenty-four hours before staining, the appearance was similar to that after only fifteen minutes in alcohol. Again, if the sections were in alcohol forty-eight hours before staining, the appearance was similar. Increasingly long periods in alcohol did not appear to alter the staining or size of the metachromatic bodies.

Sections from "normal" brains stained before treatment in alcohol showed some pale indefinite patches as in the affected brains similarly treated. Fifteen minutes in alcohol, in about 50 per cent. of the brains examined, was productive of pinkish shreds, generally considered to be derived from myelin. Such shreds or masses were more definitely pink if the sections had been immersed in alcohol

TABLE II
POINTS OF COMPARISON BETWEEN MYELIN SUBSTANCE AND METACHROMATIC BODIES

Features	" Myelin substance " bodies	" Metachromatic " bodies
Number	Usually few	Very many
Distribution	Throughout white matter	Throughout white matter
Size	Variable : mainly 10–15 μ ; few may be 100 μ	Variable : 10–100 μ ; majority 50–100 μ
Shape	Single or multilobated bodies, elongated lobated strands	Multilobated, spherical or elongated along fibre tracts.
Occurrence : Frozen section Paraffin section Celloidin	Present Absent Occasional single form	Present Present Present
Stain reactions in frozen sections : Toluidine blue Mucicarmine	Present Absent	Present Present
Bi-refrangement	Present	Present

for twenty-four hours: and these masses resembled metachromatic bodies in structure. They were still not so numerous, and were found in only about 50 per cent. of the brains examined. In sections after more prolonged immersion in alcohol, up to forty-eight hours, fewer myelin-shreds were found than in those immersed for twenty-four hours. After increasingly long periods in the alcohol the "myelin substance" did not appear. No concretions were found in the alcohol used to treat the sections, nor in alcohol in which the blocks of tissue were kept for twenty-four hours.

SOLUBILITY OF METACHROMATIC BODIES IN SOLVENTS

Method.—Frozen and celloidin sections were placed in chloroform, ether, or acetone for fifteen minutes and then stained with toluidine blue; or treated with alcohol, and then exposed severally to chloroform, ether, or acetone for fifteen minutes and then stained with toluidine blue.

Findings.—When the frozen sections were subjected to the action of one of these liquids, chloroform, ether, or acetone, before alcohol treatment and staining, none of the liquids had any apparent effect on the metachromatic bodies. When the sections were first put in one of the liquids, and then stained, the metachromatic bodies were seen to be soluble to some extent. With chloroform there was a partial solution of the metachromatic bodies immediately, but the process was not completed until some days after mounting: then "fatty" globules could be seen on the surface of the sections. With ether and, to a lesser extent, acetone, there was a partial solution of the metachromatic bodies with the production of a blurred outline. The metachromatic bodies in frozen sections stained with toluidine faded some weeks after mounting, and there was also some diffusion into the mounting medium: "Mersol" or balsam.

In celloidin sections the metachromatic bodies were far more resistant to the liquids and did not diffuse. This result was anticipated, as the bodies had already remained distinct after prolonged treatment in alcohol and ether during the embedding processes. It is of interest that the myelin material of the "normal" brains behaved in a manner similar to that of the metachromatic bodies found in the pathological brains, except that it was more readily soluble in the liquids tested.

LIPASE DIGESTION

Method.—Frozen and celloidin sections of the brains exhibiting metachromatic bodies and "normal" brains were incubated for times varying from thirty minutes to seventeen hours in a solution of lipase and sodium taurocholate buffered to a pH of 8.2 (100 ml. of 20 per cent. lipase solution and 50 ml. of 1 per cent. sodium

taurocholate). The sections were then washed, put in alcohol, and stained with toluidine or thionin, Ehrlich's hæmatoxylin and eosin, or Mayer's mucicarmine.

Findings.—It was thought that some indication of the composition of the metachromatic bodies might be gained by establishing their reaction to enzymes. It was recognized that the tissues available had already been altered by fixation, dehydration, and embedding, and that any action on them would be different from that of the enzyme on fresh tissue. Nevertheless it seemed possible that some constant reaction to an enzyme should prove of value.

It was found that in frozen sections the metachromatic bodies were mostly digested away to leave non-staining areas, possibly holes. Where digestion was incomplete the bodies had become ragged looking with a blurred outline. They stained pink with toluidine and pale purple with mucicarmine.

In celloidin sections, instead of the homogeneous staining appearance of the metachromatic bodies usually noted, there was a coarsely granular denseness in the centre of the mass, surrounded by a clear area. These granules stained deeply with thionin, hæmatoxylin and eosin, and mucicarmine, leaving a clear non-staining area around. The effect of the lipase on the rest of the section was to show up the detail of glial nuclei, and to a lesser extent the nerve cells, more clearly than usual.

In the frozen section of the "normal" brains the effect of lipase on the myelin substance was to "digest" it away almost completely. The shreds remaining were blurred or stained faintly.

The effects described above were seen most clearly after seven hours' incubation. When the sections were incubated for a shorter time they showed similar changes, but to a slighter degree; after more than seven hours the entire sections became somewhat hazy, and metachromatic masses could not be distinguished.

FIXATION FACTORS TESTED

In order to find out if any factors occurring between the death of the animal and the staining of its tissues played a part in the genesis of metachromatic bodies, a further series of brains was investigated. In these experiments brains were obtained from humans, sheep, and rats. The human material came from patients dying from causes that were primarily non-cerebral, namely reticulo-endotheliosis, tuberculosis, heart failure (2), carcinoma of stomach, carcinoma of prostate, and pneumonia. They were obtained as early as possible, and not later than twenty-four hours after death. The two sheep used were healthy; they were killed by nembutal and bleeding. The rats used were

killed by chloroform and the brains were removed and treated immediately as described below.

As it had been suggested that metachromatic bodies occurred more commonly in the heat of the summer, or when the material was fixed under warm conditions, the effects of temperature were investigated. The concentration of the formalin which we used as a fixative was investigated, and also the lapse of time between death and fixation. The effects of much handling and washing of the material was studied, and also the length of time of preservation in formalin.

Method.—One human brain and four rat brains were fixed in 20 per cent. formol saline for times varying from one to fourteen hours; they were removed from the fixative, put in the incubator at 37° C. for five hours, and then refixed in 20 per cent. formol saline. One human brain and two rat brains were also put in the incubator at 37° C. for five hours, and then fixed in 20 per cent. formol saline. Two rat brains were put in the refrigerator for four to eight hours immediately after autopsy, and then fixed in 20 per cent. formol saline (Table III). From these brains sections were prepared.

To determine the effect of formalin concentration, the following methods were adopted. Very large slices from "normal" human and horse brains were fixed in 5 per cent., 10 per cent., and 20 per cent. formalin in water (May and Baker's formalin); and similar slices in 5 per cent., 10 per cent., and 20 per cent. formol saline (formalin in 1.75 per cent. NaCl). Sections were prepared.

To study the effect of delay in fixation, five human

brains and brains from two normal healthy sheep were obtained immediately after death. In each case the brain was divided longitudinally into two halves. Throughout the experiment one half was kept in the refrigerator, the other at laboratory temperature, both in airtight containers. Large slices from each half were fixed in 20 per cent. formol saline at intervals of approximately ten hours, up to one hundred and twenty hours. Again one "normal" brain was divided longitudinally, of which one half was washed for ten minutes in tap water before fixation in 20 per cent. formol saline, and the other half was "handled" considerably before fixation in 10 per cent. formol saline. Healthy brains fixed for varying lengths of time in formalin—four days to several years—were examined.

Findings.—The effect of varying temperatures during fixation was of interest. In none of the brains submitted to the temperature variations were metachromatic bodies found.

In brains submitted to different concentrations of formalin and formol saline no metachromatic bodies were to be seen.

It was considered that if these metachromatic bodies were fixation artefacts, delay in fixation would be their most probable cause. Paraffin and celloidin sections of the brains from the delayed fixation experiments showed no metachromatic bodies. In the frozen sections there were a few of the pink bodies derived from myelin already referred to. Collected sections from other human material, where the interval between death and

TABLE III
TEMPERATURE VARIATIONS IN FIXATION OF BRAINS

Material	Cause of death	Autopsy	Fixation	Incubator	Refrigerator	Fixative
1 human brain ..	Reticulo-endotheliosis	Within 12 hours of death	For 4 hours in 20% formol saline	For 5 hours		Refixed in 20% formol saline
4 rat brains ..	Chloroform	Immediately	For 1-4 hours in 20% formol saline	For 5 hours		Refixed in 20% formol saline
1 human brain ..	Tuberculosis	Within 12 hours		For 5 hours		Fixed in 20% formol saline
2 rat brains ..	Chloroform	Immediately		For 5 hours		Fixed in 20% formol saline
5 human brains ..	Tuberculosis Pneumonia Heart failure Carcinoma of prostate Carcinoma of stomach	Within 24 hours of death			10-110 hours	Fixed in 20% formol saline
2 rat brains ..	Chloroform	Immediately			4-8 hours	Fixed in 20% formol saline

the autopsy varied from twelve to sixty hours, were also examined. No metachromatic bodies were found in these sections.

Neither handling of the brain at autopsy, nor the length of time in formalin—four days to several years—were found to be associated with the occurrence of metachromatic bodies in either celloidin or paraffin sections.

OTHER FACTORS

When the cause of death was studied as a possible factor, collected celloidin sections illustrating widely different disease conditions, mainly but not entirely of a neurological nature, were examined. These specimens included all the common degenerative, vascular, infectious, and neoplastic neurological conditions. Metachromatic bodies were not found so far in any sections other than those described in this paper. "Holes" were frequently present in the white matter, but there was no staining in these areas. In many cases the appearance was that usually associated with oedema.

It was considered possible that metachromatic bodies might arise in a consequence of disordered metabolism resulting in a necrobiotic phenomenon. To investigate this aspect of the problem sections from the brains of patients dying in a state of coma, anoxæmia, starvation, or dehydration were prepared and examined. No metachromatic bodies were seen.

Again, collected sections of brains of patients varying in age from infancy to eighty years at death were examined. No metachromatic bodies were seen. The age of the patient with Huntington's chorea was fifty-seven. The horses were young adults.

Brains of mice, rats, guinea-pigs, ferrets, and sheep were examined without showing more than the occasional pale form of metachromatic body already described in the normal brain.

Discussion

Sections from the brain of a man with Huntington's chorea and from the brains of two horses dying from grass sickness were prepared in celloidin, in paraffin wax and by freezing. In the three cases frozen and celloidin sections of all parts of the brain contained very large numbers of metachromatic multilobate masses. These masses were limited to the white matter, extending into the grey only in association with fibre tracts.

The masses were clearly the substance described as "mucocytes" by Grynfeldt. They were most numerous throughout the cerebrum and cerebellum, and less plentiful in the brain stem and medulla. They practically equalled the number of glial nuclei

in the field (Figs. 1 and 3). Many seemed to be formed by the conglomeration of separate bodies which produced a multilobate mulberry effect (Figs. 2 and 4). This lobulated appearance was present even when the masses remained unstained. The diameter varied between 10 and 150 μ , with the majority between 100 and 150 μ . Where the mass was more elongated than rounded, the long axis was invariably directed along the length of the nerve fibres, and never transversely. No nuclei or remains of nuclei were seen in the masses; when glial nuclei appeared to be involved in the masses they were optically readily separable from it. In view of the very large number of metachromatic bodies present such a superficial association between glial nuclei and the bodies was inevitable. In the paraffin sections the areas were markedly larger than the metachromatic bodies in celloidin sections, and fewer in number (Figs. 5 and 6). In the horses no other abnormality was observed: vessels, neurones, axis cylinders, and myelin sheaths all appeared normal. There was no increase in glial cells; this was confirmed by nuclear counts in similar areas of affected and normal horse brains. There was no change in the oligodendroglia, and no staining of the cytoplasm of these cells with muci-carmin. In the brain of the case of Huntington's chorea the pathological changes were confined to the grey matter. They were only those associated with the condition.

In "affected" brains metachromatic bodies appear in very large numbers. They can be shown to stain with basic dyes, and may therefore be assumed to be acid in nature. They stain metachromatically with thionin and toluidine, and stain with muci-carmin; they contain, therefore, substance or substances similar to those found in mucin. They do not give the stain reactions of fat, glycogen, or amyloid. Before staining, treatment with alcohol is necessary to show up the metachromatic bodies in a definite form. They are to some extent soluble in chloroform, acetone, xylene and ether, but they are sufficiently insoluble in ether to remain present during the clearing and embedding processes in celloidin and paraffin techniques. In frozen sections they are bi-refringent, unless acted on by alcohol forty-eight hours or longer. They are also bi-refringent in paraffin sections. The possibility of "metachromatic" bodies being entirely due to fixation has been considered. If, as some observers have thought, they are due to fixation artefacts only, it should be possible to produce metachromatic bodies by reproducing the conditions of fixation under which they occurred. A wide range of variations in the technique of fixation has been used, but no factors were found which

produced these bodies. The cause of death, the condition before death, and also age and species specificity have been examined; these factors could not be related to the presence of the metachromatic masses.

The superficial resemblance between the metachromatic masses and the mucoid degeneration present in certain forms of Schilder's disease was observed, but many differences must be noted. In these cases of Schilder's disease there are marked changes which include degeneration of myelin sheaths, mucoid degeneration of oligodendroglial cells, and a possible presence of compound granular cells. There were no such gross alterations in the brains described here. The appearances of the "metachromatic bodies" and of the mucoid degeneration in such a case of Schilder's disease have some resemblance when stained by thionin, but the use of a trichromic stain such as azan reveals the "metachromatic bodies" as pale pink or colourless, as opposed to the definite pink and blue mucoid masses in such forms of Schilder's disease. Corpora amylacea cannot be confused with the "metachromatic bodies." They may be present at the same time, and the corpora amylacea can be seen to be more spherical and solid, and to present different staining reactions as they are not metachromatic.

In frozen sections of "normal" brains shreds of a material may be seen, which are generally considered to arise from myelin. These also stain metachromatically with thionin or toluidine, but do not stain with mucicarmine. They are soluble in chloroform, ether, acetone and xylol, and are bi-refringent. They do not appear in paraffin sections, and only very occasionally and sparsely in celloidin preparations. The resemblance between the form and character of the metachromatic bodies, and the myelin-derivatives in normal brains, is too marked to be accidental. The main differences between the ordinary myelin derivatives and the metachromatic bodies are the greater number of metachromatic bodies compared with the few myelin shreds in frozen sections, the persistence of the metachromatic bodies in paraffin and celloidin preparations, and the fact that they stain with mucicarmine (Table II).

It may, then, be reasonably assumed that the metachromatic bodies are derived from the myelin, or from the ground substance of the brain. This view is strengthened by the observation that the acid reaction of the bodies, and their specific reactions with toluidine and mucicarmine are like those of polysaccharides and their derivatives, particularly those that are sulphated, present in mucus, cartilage, and other intercellular structures. The fact that

they appear only after treatment with alcohol suggests that a disturbance in the colloid disperse suspension of the complex compounds consisting of tri-glycerine, carbohydrates, and nitrogenous substances present in myelin, must be brought about first. A pre-existing disturbance in these substances may explain an alteration in the number and character of the metachromatic bodies. The most important change would seem to be the solubility of the myelin derivatives: the more soluble is the myelin in shreds, the less soluble is the substance found as metachromatic bodies.

Further investigations are being made on a similar line, with particular attention being paid to obtaining fresh material of varied disorders and diseases. The examination of such tissues at set times after death and fixation, and immediately after staining, standardizes the result. Substance present in the freshly prepared slide may disappear in the course of time. The hydrogen ion concentration of the tissue examined, or of a reagent used in the preparation of sections, may contribute to the appearance of metachromatic bodies.

Conclusions

Metachromatic bodies are considered to be derived from myelin or from the ground substances of the brain; they are less soluble in alcohol, ether, chloroform or acetone, than myelin, and share characteristics with mucus and similar substances.

At the present stage of investigation there is nothing to show that the metachromatic bodies are entirely artefacts, in view of the inconstancy of their occurrence, and the failure to produce them deliberately by varying laboratory technique. The process leading to the formation of these bodies is diffuse, affecting all or the greater part of the white matter in the brain; but a pathological agent or patho-physiological process leading to their appearance has not been found.

It is suggested that for the production of manifest metachromatic bodies the following conditions obtain: an alteration of solubility of myelin or the ground substance, arising from metabolic factors before death, or resulting from factors in the time after death. A minor variant in the treatment of the material after death cannot be excluded as being contributory.

Summary

Three brains exhibiting "metachromatic bodies" were examined.

"Normal" brains were subjected to different technical procedures to find out if such metachromatic bodies were fixation or staining artefacts.

A search was made for metachromatic bodies in brains from a large variety of conditions.

The origin of metachromatic bodies was discussed.

My thanks are due to Dr. E. Arnold Carmichael, in whose department these investigations were carried out, for his help in writing this paper. I also wish to thank Dr. J. G. Greenfield for permission to use his material and for his advice; Professor Russell Greig for his material; and Dr. Peter W. Nathan for his helpful criticism. I am also grateful to Mr. James Mills for his generous help with technical problems, and to Miss Vera Burgess for her able co-operation.

REFERENCES

- Anderson, J. (1929). "How to Stain the Nervous System." Livingstone. Edinburgh.
- Ansalone, G. (1924). *Riv. sper. di freniatria*, **48**, 208.
- Bailey, P., and Schaltenbrand, G. (1927). *Dtsch. Z. Nervenheilk.*, **97**, 231.
- Bielschowsky, M. (1927). *Zbl. ges. Neurol. Psychiat.*, **47**, 604.
- Buscaino, V. M. (1920). *Riv. patolog. nerv.*, Firenze, **25**, 197.
- Buscaino, V. M. (1922). *Ibid.*, **27**, 641.
- Ferraro, A. (1926). *Arch. gen. neurol. psych. psicoanal.*, **7**, 183.
- , A. (1928). *Arch. Neurol. Psychiat.*, Chicago, **20**, 1065.
- Foot, N. C. (1933). *Stain Technology*, **8**, 101.
- Grinker, R. R., and Stevens, E. (1929). *Arch. Path.*, **171**.
- Grynfeldt, E. (1923). *C. R. Soc. Biol. Paris*, **89**, 1264.
- (1926). *C. R. Ass. d'Anat.*, 21st Reunion. March 29–31.
- (1927). *Bull. Soc. Sci. med. biol. Montpellier*, **450**.
- , Pélissier, G., and Pagès, P. (1924). *Montpellier Med.*, **46**, 260.
- Lhermitte, J. J., Kraus, W. M., and Bertillon, F. (1924). *Arch. Neurol. Psychiat.*, Chicago, **12**, 620.
- , and Radovici, A. (1921). *C. R. Soc. Biol., Paris*, **84**, 931.
- Pélissier, G. (1924). "Syndrome Wilsonien consécutive à la névrite épidermique. Contribution à l'étude de la dégénérescence mucocytaire de la névroglie." *Thèse de Montpellier*. Imprimerie l'Abeille.
- Penfield, W., and Cone, W. (1926). *Arch. Neurol. Psychiat.*, Chicago, **16**, 131.
- Salustri, E. (1924). *Riv. sper. di freniatria*, **48**, 255.