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1. Wells P. Eagleton, M.D., Brain Abscess (its surgical pathology and operative technic) Newark, N. J.: The Macmillan Company. 1922. p. 157.
2. Frederick P. Moersch, M.D., & George P. Sayre, M.D., Neurologic Manifestations associated with dissecting aortic aneurysm. The Journal of the American Medical Assn., Vol. 144, No. 14: December, 1950. 1145-1146.

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NEUROLOGY

Vol. III JANUARY 1955 No. 1

EXPERIENCES IN THE MANAGEMENT OF INTRACRANIAL TUMOURS*

(Based on A Series of 68 Consecutive Verified Cases from 1951 to the end of 1954)

by R. G. GINDE, Bombay.

The Honorable Chief Minister, Pandit Shukla, distinguished guests, fellow colleagues, ladies and gentlemen,

On behalf of the Neurological Society of India and on my own behalf, I would like to express my grateful thanks to the medical fraternity of this city, and particularly to Professor Berry of the Medical College, Nagpur, who has been virtually responsible for our presence here today.

Since the inauguration of our Society 3 years ago, my distinguished predecessors have addressed these annual meetings on "Neurology Coming To Life", "Creative Neurology" and on "Neurological Landscape". Although I have been interested in this field for some years past, I have only been able to devote my time exclusively to this speciality for a little over a year. Last year, I suddenly found myself elected to this high office, when I had just been admitted as an ordinary member of our Society; I am grateful to my colleagues for the honour done to me. But, I am neither mature in years, nor do I possess a vast experience upon which I could draw. So, instead of making a philosophical or prophetic speech, I have thought of presenting before you, on this occasion, my experiences in the management of intracranial tumours from 1951 to the end of 1954, based on a series of 68 verified cases treated surgically.

Harvey Cushing, the architect of modern neurological surgery, observed as early as 1932, that "Neurological Surgery is a large subject, and the surgery of brain tumours is a special field in it. Indeed, the day may well enough come when certain surgeons will find enough to keep themselves fully accupied by attending exclusively to tumours of a single type. This prophetic utterance has already started materializing to a great extent in the western countries, where there is a very large number of well-trained neurological surgeons working, with their team of coworkers in the same and allied fields, with all the modern equipment and facilities with the result that this speciality has been taken to its present high pedestal, to which more and more young men are being attracted every day. Whereas in our vast country with its teeming millions, neurology and neurosurgery have been practised only since the last few years, there are only a few such specialists who have to handle all the cases, which are now making a regular bee-line for relief.

I shall briefly narrate the development of this speciality at the Seth G. S. Medical College and the K. E. M. Hospital, Bombay. In our country, more or less similar conditions probably exist in most of the medical colleges; and a beginning to develop this speciality could also be made in some of the more fortunate ones. Till recent years, comparatively very small amount of neurological and neurosurgical work was found at the hospital, and all of it was done by general physicians and surgeons. Gradually, with increasing interest, more and more cases with neurological disorders began to be operated — starting with surgery of the quick surgeons, they all became unsuccessful. The reason is obvious. The surgeons then had to face lack of properly developed anaesthesia, blood transfusion and adequate asepsis during operations. Moreover they had not the present advantages of chemo-

*Presidential Address delivered at the Fourth Annual Conference of the Neurological Society of India, held at Nagpur, M.P., on 4th February, 1955.

therapeutic and antibiotic agents which the peripheral nerves, sympathetic nervous system, spinal cord and nerve roots, and the brain itself. Although stray attempts were made to operate on brain tumours almost from the very inception of the hospital, in 1926, unfortunately in spite of the best efforts of these experienced, skilful and present surgeons have in ample measure. These factors impeded progress in this branch of surgery even in the West. All this had made a deep impression on my student mind. I was very happy when Drs. Cooper and Baliga, both my teachers, showed some real success in this field in 1948, even while working as honorary general surgeons. Their results have been presented before the annual meeting of the Association of Surgeons of India in 1948. Since 1951, almost till the end of 1953 — although I had to do certain amount of general surgery both in private and at the hospital, where I also am working in the honorary capacity — my colleagues very kindly allowed me to handle all cases of intracranial tumours, for which I am grateful to them.

Even during this short period, the neurological work coming to us — at our departments of neurology, neurosurgery and psychiatry — is far more than what my colleagues and I can cope up with in the present set up with reasonable urgency and safety. As a result, it has been possible to treat only 68 cases of intracranial tumours. However, if this figure is compared with the 194 tumour cases that were verified, either at operation or autopsy, by Cushing in his Hopkins period of 1901 to 1913, there is no cause to get disheartened.

In presenting my experiences with these tumours, I shall avoid giving statistical figures for comparison with those of other workers, as the series is small, the patients are seeking relief — as you will later see — at a late stage of the disease and the facilities for work are not such as one would like to have. Even so, there have already been some unusual findings, which have been of great educative value to us, and some of which you might find interesting.

Although brain tumours occur approximately with equal frequency in the two sexes, in the present series there have been 43 cases in the males as against 25 in the females. This may be partly due to the fact that there are more hospital beds for male patients, and partly due to the fact that our women are reluctant to go to the hospitals, and also because of some difficulties which they have to face.

As may be seen from the accompanying chart, showing the age incidence, the vast majority of these tumours viz. 55 cases or 80 percent of them occurred in persons in the very active and productive period of their lives, between 10 and 50 years of age. It may be noted that in spite of the very small number of paediatric beds at the hospital, relatively more cases occurred in the younger age group. The youngest patient in this series was a boy of 3 years and the oldest, a man of 67. In all, 56 tumours were in adults and 12 in children. 43 tumours were located above the tentorium, of which 4 occurred in children, and 23 were found under the tentorium in the posterior fossa, of which 8 were in children, — a finding which is in accordance with the general experience all over. Two of the cases were located in the brain stem, and were extending from below the tentorium on to the hypothalamic region. These have been designated as supra and infratentorial in location.

TABLE I

TABLE SHOWING AGE INCIDENCE OF 68 INTRACRANIAL TUMOURS

Age In Years	No of Cases	
0 — 10	7	
11 — 20	16	55, or 80%
21 — 30	16	
31 — 40	14	
41 — 50	9	
51 — 60	4	
61 — 70	2	

The 68 tumours have been classified pathologically as follows:— (See table).

TABLE II

I	Glioma (varia)	15
II	Pituitary tumours	3
III	Meningiomas	15
IV	Acoustic Neurinomas	8
V	Congenital tumours	9
VI	Granulomas	10
VII	Blood vessel tumours	1
VIII	Miscellaneous	6
IX	Unclassified	1
	Total	68

Of 15 Gliomas, there have been 5 glioblastomas, 5 astrocytomas, 2 ependymomas, 2 medulloblastomas and 1 unclassified glioma (probably a subependymoma). All the five glioblastomas were extensive, three were supratentorial — of which two arose in the frontal lobes, extending on to the basal ganglia, the opposite side and to the hypothalamus. The other two occurred in the brain stem and were very extensive, extending from the medulla to the hypothalamic region.

All the five astrocytoms were supratentorial four of which occurred in the cerebral hemispheres, and the remaining in the posterior hypophysis as an intrasellar lesion; this rare case was reported last year. Only one of them was cystic with a large mural nodule, and it was in the right frontal lobe.

The two ependymomas were both infratentorial and were situated in the roof of fourth ventricle. One of these showed many mitotic figures and has been designated as ependymoblastoma. There were two medulloblastomas, both in the posterior vermis — one in a child and the other in an adult boy. The former died 2½ months following the operation, and the boy was given Deep X-Ray therapy and is alive and well, now for over 18 months. There was one case of glioma in the left lateral paraventricular region, near the atrium in the occipital lobe, which has not been finally classified as yet, but probably it is a subependymoma.

The three pituitary tumours were all adenomas of which two were verified as chromophobe adenomas and the third, one of the early cases, could not be verified histologically as, I regret to say, the biopsy material remained unfixed being kept only in saline overnight. (Naturally it was reported as necrotic tissue by the pathologist.)

The 15 meningiomas have formed an interesting group. A detailed consideration of this group is beyond the scope of this paper. 9 of them were in females and 6 in males. The first case of meningioma arose from the dura near the sigmoid sinus in the left posterior fossa. It was a large tumour weighing 120 grams. The remaining cases were found arising, four from the parasagittal parietal region, 3 from the pterional region, one from the left side of the falx in the frontal region, one from the floor of the middle fossa, one from the middle third of sphenoidal wing, another, a recurrent tumour from the orbit — probably arising from the dural sheath of Schwalbe, a case of meningioma-en-plaque arising from the lesser wing of the sphenoid, and a very large hyperostotic meningioma from the right frontal region. Besides these there were two rare sites of origin of these meningiomas, one was arising in the suprasellar region and the other definitely from the roof of the right orbit.

I will mention here only 2 of them in unusual locations. One was arising from the meninges over the roof of the right orbit and assumed the size of a tennis ball. This is a very uncommon site, as none was found in a series of 334 cases recorded by Cushing and Eisenhardt, although they have mentioned this site in the experience of other workers in their monograph. The second case was a suprasellar meningioma, which I failed to diagnose for over 2 years, although the patient went on knocking at us, complaining of headaches and progres-

sive dimness of vision; his visual fields showed rather bizarre pictures. His fundi showed slight pallor; air studies were not done earlier as the plain X-Rays of his skull did not reveal any evidence of rarefaction, vascularity, density or calcification and the sella was quite normal. Besides there were many advanced cases with intense papilloedema on hand which needed urgent attention. It is unfortunate that he became almost blind before our very eyes, when a simple pneumogram by the lumbar route revealed the nature of the lesion, which was finally verified at operation. This man is now doing well.

Of the 8 acoustic neurinomas, 6 were found on the left side and 2 on the right. Unlike the meningiomas, 6 of them occurred in the male and only 2 in the females.

The congenital group of tumours consisted of 6 craniopharyngiomas, 2 chordomas and an epidermoid tumour. Of the 6 craniopharyngiomas, 4 were very large, extending in the frontal, temporal and parietal lobes; 1 was medium sized. These were mainly suprasellar and 4 of them showed well marked calcification. The sixth case was entirely intrasellar and cystic, and showed no calcification at all. All of them contained crystals of cholesterol and 5 of them showed well marked lining of squamous stratified epithelium.

Both the chordomas were very large and showed cystic degeneration; one occurred in a middle aged woman in the right middle fossa with extensive calcification and the other occurred in an elderly man of 60, in whom it produced complete destruction of the bones around the left anterior temporal region and had produced a temporal swelling, marked exophthalmos with blindness and pseudo-ophthalmoplegia. Histologically this showed evidence of malignancy. Both these patients are well so far.

The solitary epidermoid tumour — tumour perlees of Cruveilhier — occurred in a young adult male nurse of 25 in the left cerebello-pontine angle. He was operated nearly 2 years ago. His facial nerve could be saved; he wrote a month ago stating that he was well and still working in the hospital.

Among the 10 granulomas, one was a large gumma which was situated in the right temporal lobe and was adherent to the dura. He was operated after he had been in coma for over 12 hours with dilated fixed pupil on the same side which was thought to be due to tentorial herniation, as he developed this and became comatose after a lumbar puncture in the medical wards the previous day. It is a pity that this man died on the 12th postoperative day after partially coming out of coma due to meningitis and cerebral edema. The other nine cases were all tuberculomas. These lesions were large weighing 38 to 45 grams. 7 of them occurred in the males and 2 in the females. 6 of them were infratentorial, mostly situated in one cerebellar hemisphere; of the remaining three, 2 were in right parasagittal parietal lobe and the third in the left frontal lobe. Note the relatively high incidence of tuberculomas, which is undoubtedly due to the still high incidence of tuberculosis in our country. Mention may be made here that although these patients do fairly well following surgical removal of the lesion, they finally succumb either to tuberculous meningitis or recurrence of the pulmonary focus while they are still in the hospital or after their discharge. This seems to be due to poor nutrition, want of development of immunity, drug resistance, leading to flaring up of the original focus.

The miscellaneous group comprises 2 osteomas, one in the left parietal and the other in the occipital bone extending from the inion almost to the foramen magnum. There was one case of a large hemangioma in the right parietal bone, another of a large hemangioendothelioma of the parieto-occipito-mastoid region, a case of a moderate sized colloid cyst of the third ventricle and one calcified tumour, whose pathology could not be made out histologically. There was one large hemangioblastoma in the right parasagittal region in the frontal lobe and one angioblastic unclassified tumour which occurred in the midline of the vermis of the cerebellum in a boy of $6\frac{1}{2}$ years.

The duration of symptoms have not borne any consistent relation to the size and nature of the tumours as seen at operation.

The symptomatology has been as varied as has been described in practically all the standard works on the subject, and has included such diverse symptoms as headache, vomiting, impairment of vision going on to complete blindness, seizures of one type or another, weakness or paralysis, unsteadiness of gait, various types and combinations of cranial nerve palsies (especially the extra-ocular group, often with ophthalmic division of the fifth, or fifth, sixth, seventh and eighth, or bilateral sixth with a seventh etc.) tremors, drowsiness, yawning, unconsciousness, incontinence of urine, alteration in personality and behaviour, difficulties in speech, swelling on the head, proptosis, pain in the nape of the neck, enlargement of the head, stunted growth with hypogenitalia, obesity etc. As no useful purpose will be served by such a mere tabulation of the symptoms, some of the common groups of symptoms which may give an idea of the incidence and stage of the disease have been considered in some detail. Thus, 60 out of 68 cases, or over 88 percent of them had two or all of the symptom-triad of headache, vomiting and impairment of vision. The ophthalmological findings, especially the appearance of the fundi in these cases were carefully scrutinized and have been tabulated as follows:— (See table).

TABLE III

TABLE SHOWING APPEARANCE OF FUNDI IN 68 TUMOUR CASES

Fundal Changes	Normal	E.P.	P.O.A.	B.P.	S.O A.	Total Blindness
No. of cases	5	8	9	19	17	10

Key Table
E.P.: Ear y Papilloedema, or Early Pallor.
P.O.A.: Primary Optic Atrophy.
B.P.: Bilateral Papilloedema.
S.O.A.: Secondary Optic Atrophy.

Note that only 5 of these 68 cases had normal fundi. In the West, where neurological surgery is well established, nearly a third of the tumour cases has normal fundi at the time of operation — an important factor in the present low mortality of these cases. In the present series, 55 cases or over 80 percent of them had marked changes in the fundi; 17 cases showed secondary optic atrophy and 10 total blindness.

Seizures of focal or generalized types occurred in 15 of them (22%). Furlow and Sachs in an analysis of 724 cases of brain tumours in 1936 found the presence of seizures of one kind of another in 20.7%. 20 cases had already hemiparesis or hemiplegia (29.4%). 7 had incontinence of urine at the time of admission. 11 patients were apathetic and|or drowsy; and in addition, there were 2 in deep coma, in whom tentorial herniation was strongly suspected. One patient was very excitable and four others showed alteration in their behaviour. It is no wonder then that a very large percentage of these patients were clinically diagnosed. The advanced stage of most of these cases, however, has affected the total operative mortality of the entire series.

It is well known that operative mortality increases with progressive increase in the intracranial pressure. Frank Nulson from Dr. Francis Grant's clinic in Philadelphia has recently reported in 1950, that mental dulling also increases the operative mortality of intracranial tumours. In a series of 200 consecutive cases, he found that in alert patients the mortality was about 12 percent, and when the patients were dull, it rose to 42 percent, when stuporous to 61 percent and in comatose patients the mortality went up to 89 percent.

Diagnosis:

In the present series, the presence of an intracranial tumour was clinically diagnosed in 55 cases and strongly suspected in the remaining 13. In 38 of them the site of the lesion was definitely localized and in 16 others it was strongly suspected. In 14 cases, the site of the lesion could not be

made out on clinical grounds alone. As regards, the nature of the lesion, this was correctly guessed in 36 of them and could not be made out in the remaining 32 cases.

X-Rays of the Skull :

Plain X-Rays of the skull were taken in 67 cases ; and in 57 of them, they showed evidence of increased intracranial pressure by way of decalcification or destruction of posterior clinoid processes and the dorsum sella, flattening of the sella, separation of sutures, enlargement of the head and beaten-silver appearance. In 32 of these films, localizing signs by way of erosion, vascularity, normal and|or abnormal calcification, complete destruction, hyperostosis, etc. were detected. Of these 32 cases, the probably nature of the lesion was suspected in 22. In only 10 cases the plain X-Rays showed no evidence of increased intracranial pressure, and were regarded as normal radiologically. These findings fit in very closely with the 55 cases which showed varying degrees of changes in the fundi from early papilloedema to varying degrees of primary or secondary optic atrophies.

The demands made on this method of investigation in the diagnosis of neurological disorders since its discovery have been overwhelming. By careful and painstaking studies, it is possible to diagnose or at least get a strong clue to the presence and site of the tumour in 60 to 80 percent of the cases. The E.E.G. department of our hospital with its single machine, the very bare minimum of staff and heavy demands made on it by a large number of waiting patients, could not be of much help in the diagnosis of intracranial tumours in spite of the enthusiasm and cooperation of all its members. It was possible to utilize this investigation on only 16 cases, as in a good few of them, the diagnosis was quite apparent from clinical and plain X-Ray studies. It was suggestive of a lesion in only 6 of them. With better facilities in its working and further experience, it is hoped to get a more reliable evidence from this method of investigation.

Cerebral Angiography :

Again, owing to non-availability of special equipment, adequate personnel and facilities for work, it was not possible to employ cerebral angiography as extensively as one would have like to do. However, even in the small series of 11 cases, where it was carried out with the ordinary 500 M.A. diagnostic X-Ray machine, it was helpful in giving us the site and the probable nature of the lesion in 10 of them. Being a safer procedure than air studies, not requiring to be followed immediately by an operation, being less trying to the patient and giving the patient and his relatives a better chance of taking a decision, it should be employed more and more, as has already been done in practically all the advanced countries in the world. It gives strong clues to localization and nature of the disease, helps in avoiding operations on advanced and hopeless cases like glioblastomas. Besides, it is the sole method by which intracranial aneurysms and blood vessel malformations can be demonstrated. It is hoped that this method also becomes available to a much larger number of our patients.

Air Studies :

It is well known how the discovery of cerebral pneumography by Dandy has revolutionized the diagnosis of intracranial tumours. Depending on the care and attention to details given to the problem, it has been found helpful in diagnosis in 80 to 97 percent of intracranial tumours ; so much so, that even today it is much more reliable than all the other methods combined. The procedure is relatively simple, but is time consuming and rather trying to the patient. The only danger and rather a grave one at that, is, that it is likely to be followed by a 6 to 10 percent mortality if it is not almost immediately followed by a craniotomy. Therefore, air studies, especially ventriculography, which alone should be done in the presence of increased intracranial pressure, should be done on the threshold of the operation theatre, so that a craniotomy could be done almost immediately following

it. In the present series, air studies were done in 51 cases, and it was possible to arrive at a preoperative diagnosis as regards the location of the lesion in 50 of them. In the remaining case also, although the tumour was missed at operation, the ventriculographic findings were found to fit in with the tumour as demonstrated at autopsy. Pneumography by the lumbar route was employed 7 times in cases where there was no evidence of intracranial hypertension and ventriculography in 44 cases. In four of these latter cases pantopaque or myodil ventriculography was also done using 2 to 3 c.cs. of the radio-opaque oil.

Operative Diagnosis :

These 68 cases from the K.E.M. and other hospitals, were operated in all 80 times. 7 cases were operated a second time weeks or months later, another case 3 times and 1 case four times at intervals of about a year in between the operations. The lesion was demonstrated and removed as radically as possible in 64 of them. The type of lesion was verified by histological examination in 61 of them. (In one of the early cases of a large intrasellar tumour, which grossly looked like a chromophobe adenoma, the histological report came as necrotic tissue. The tumour material which was removed at the time of operation, I regret to say, remained unfixed overnight.) In the second case where a gliomatous cyst with the mural nodule and surrounding brain was removed from the right frontal lobe, no definite diagnosis of a glioma was reported from the section and more study of the tissue could not be made as it was later found that the remaining portion of the tumour material was not preserved. I may add here that it is always desirable to keep all the pathological material intact. It helps further studies. In western countries attempts are usually made to preserve such material as long as possible for further study in the light of new advances. In the third case, again an early case in the present series, of meningioma-en-plaque with hyperostosis in the lesser wing of the sphenoid bone, the thickened pieces of bones did not show conclusively the presence of meningothelial cells, although the sections were very suggestive. In three cases in the entire series, the tumour could not be found at the time of operation, but was demonstrated at autopsy. In one case, the solitary case in the whole series only a palliative operation of ventriculocisternostomy was done as the case was an advanced tumour in the brainstem, which was later confirmed by autopsy. All these details are mentioned to draw your attention to our mistakes and to remind us for exercising greater care and vigilance in the management of such details, than has been done.

I shall now deal with the operative results in this series. However, before presenting them, with your permission, I will quote certain passages from the highly critical and monumental work of Harvey Cushing on intracranial tumours : " Unless one somehow makes a game of his daily operative tasks, they become highly irksome — and some games are scarcely worth playing unless one keeps a score. In Golf, for example, it is only by the score that a player can tell whether his game is improving or falling off ; and only when his competitors keep score by the same standards, can it be told by comparison of figures whose game is the poorer. And when it comes to match play, it's safe to have onlookers count the strokes lest one of the contestants conveniently fail to remember having lifted a ball out of an unplayable ' lie '. Perfectly honest and well meaning persons have been known to cheat themselves in games of solitaire, but they are less likely to do so when someone is looking over the shoulder."

In calculating mortality percentages certain uniform standards have to be followed and fortunately these also have been already laid down for us by Harvey Cushing. " Every death in the hospital following an operation from any cause whatsoever, no matter how long the interval, is recorded as a postoperative fatality." The operation is said to have begun at a time when the incision in the scalp is taken

as a part of the craniotomy. We all know that because of the advanced stages of the disease at which patients reach us, and often over long distances, the presence of poor nutritional states and deaths that may occur from intercurrent diseases, the mortality figures will look much higher than what they actually should have been. But till these factors are overcome by medical and general enlightenment, better public health measures, better economic conditions, the provision of suitable convalescent homes, we have got to render them whatever service is possible under the present circumstances. To these, must also be added, the increased mortality due to the want of adequate medical standards, by way of provision of essential facilities in personnel and equipment, as also the presence of an unchanging team over a long period of time. Finally, it is common knowledge that the rate of mortality of cases handled by a surgeon is bound to be higher in the earlier years "owing to his relative inexperience", than that of the cases with which he deals in later years. Leave aside the tumours. Let us not forget that the mortality in cases in which the Gasserian ganglion was removed for the relief of trigeminal neuralgia was so high about 25 years ago that it was described in many textbooks as the most dangerous operation in surgery. Yet, today it is carried out with an extremely low mortality.

Besides this, one should not forget another aspect, a very important one in our vocation, and that is, the development of this speciality and the training of younger men in our country. In this connection, I would like to narrate an event that happened to the father of neurological surgery that is Sir Victor Horsely himself. A patient with a pituitary tumour was going blind and was suffering from intense headaches. Horsely on being called in consultation announced that he would operate on the patient. Whereupon his colleague remarked, "Well, Victor you know the patient will die". To which Horsely answered, "Yes probably, but if I don't do this, the men who are coming after me will never learn to operate on pituitary tumours." How right and forsighted he was, for, it is due to his pioneer work, and that of Harvey Cushing and others that we are today able to operate successfully on different types of brain tumours. In my own case, it is entirely due to the inspiration and encouragement given to me by my own teachers and colleagues in our own country and abroad, that I have been able to save the lives of some of these unfortunate patients, and make comfortable those of others. I have great pleasure in mentioning the cooperation and help of my colleagues, Drs. E. P. Bharucha, Vahia, Iyer and Dastoor and the sincere and loyal devotion to duty of my residents and nursing staff without whose assistance, even this work would not have been possible at all.

In the small number of cases, as described here, consisting of a heterogenous group, mortality figures by themselves do not really convey much meaning. Besides, as is well said in a recent paper of Dr. F. C. Grant et al in 1951, based on a series of 891 verified cases of intracranial tumours, as far as gliomas are concerned, the surgeons luck in exposing a lesion of the proper type favourably placed is more important than his skill. Pituitary tumours, cerebellopontine angle tumours and meningiomas require surgical skill, experience and also luck. However, as there is no other way for comparison, with large number of cases of other workers and with those of our own coming at a later stage, I have decided to record them as such. Out of 68 tumour cases, there were in all 26 deaths, giving a case mortality of 38.2%. These have been operated 80 times, as mentioned above, giving an operative mortality of 32.5%. On further analysis, it was found that 4 of these practically died on the table, 15 died within 2 weeks of the postoperative period and 7 died later during their stay in the hospital before discharge, due to recurrence of the lesion or other complications. I may add here that in 7 of these 26 deaths, the deaths were due either to the operation being performed on patients who were in a very desperate condition, against my own judgment as a last chance, or it was due to other causes like cessation of respiration before the craniotomy was started, (this patient went through the operation without regaining her spontaneous respiration, was kept in a respirator, and lived for 40 hours after the operation) leakage of blood on to the floor due to slipping of the canula which remained unnoticed, transfusion incompatibility leading to anuria, hyperthermia which remained unattended until a very late stage etc. All these fatalities occurred in the first few cases, but cast their heavy shadows on the total mortality figures. If they are excluded, there would have been a case mortality of 28% and an operative mortality of 23.7%. Even then, these figures are much higher than the present overall mortality of intracranial tumours in western countries, which is around 10 percent. With increasing facilities in the working conditions, with the provision of essential and adequate equipment and the continued services of vigilant, loyal and unchanging medical and especially nursing personnel, I am certain that much improved figures will be forthcoming in our country than those presented before you today.

Although it is possible to verify the nature of the lesion during the operation in most of the cases, the cause of death, whether due to surgical error or due to the nature and course of the disease is found out in the postmortem room. I shall only touch this aspect in a passing way on this occasion though it is extremely important for proper and complete evaluation of this study. In the present series, which also consists of 14 cases done in private and other hospitals, autopsy was not available in the 6 patients that died following operations. Out of the 54 cases which were operated at the K.E.M. Hospital, 20 patients died following the operation. Out of these, autopsy was available in 16 of them, i.e. 80 percent. The cause of death was determined in 13 of them and in 3 others the tumours which were not found at operation were demonstrated. I would like to make a plea here that autopsy rate in our country among the patients dying in the teaching hospitals is very low, between 20 to 25 percent, in my institution in Bombay. It may be much lower elsewhere. However, from our experience, I may suggest that by looking after our patients with sympathy and kindness throughout our contact with them, it is possible to improve upon these figures. It will indeed be another step in improving the standard of medical care and medical education, both of the teachers as well as of the taught.

I hope to present the follow-up results of the surviving cases on another occasion. Time alone will prove whether the surgical interference in these cases has been a mere apologetic peck or an attempt at giving a radical cure or maximum relief to them. Before I close, I would like to appeal to all my colleagues and others interested in the field of neurology to encourage as many youngsters as possible in this ever-widening new field and also start a tumour registry, where it would be possible to collect and record the neuropathological material with case data coming from different workers in the country.

AMINO ACID ANALYSIS OF NORMAL CEREBROSPINAL FLUID BY CIRCULAR PAPER CHROMATOGRAPHY

by BALDEV SINGH, JACOB CHANDY and DOROTHY JOSHUA, Vellore.

Introduction :

The original conception of paper chromatography may well be attributed to Pliney[17]. He used papyrus impregnated with an extract of gall nuts for the detection of ferrous sulphate. Runge, Schorubein, Goppe-sroeder[10] et al were some of the workers who contributed to his conception from 1850 to 1910. Weil[23, 24] and his associates submitted their work on this subject during the years 1950, 1951. The present day popularity, however, is due to Martin, Consden, Gorden and Synge, [2, 3, 4, 5, 6, 7, 12, 13, 14, 15] who have brought paper chromatography to be recognised as a method of scientific investigation of great use in biochemistry. Its application for separation of amino acids is attributed to Neurberger. He was interested in separating the neutral amino acids, and observed that the partition coefficients of acetylated amino acids between water and an immiscible organic solvent differed for the various amino acids. Martin and Synge used silica gel as an inert support to hold the water phase and to pass the immiscible solvent through it. Silica gel was subsequently replaced by filter paper which served to reduce the quantity of materials needed and to detect amino acids directly on the paper by treating it with ninhydrin without acetylation of these acids. Circular paper chromatography was used by Rutter[20] in 1950 to separate dyes from inorganic substances. It's use for the detection of amino acids particularly has been introduced and popularized by Giri[9] and his associates. They have made the procedure practical enough to be made use of in clinical and biochemistry laboratories of medical institutions for the study of amino acids in biological material in health and disease.

Although it was originally believed that the paper functions primarily as a support and hence the name paper partition chromatography, it, however, now is known and accepted that there are three reactions going on simultaneously during the procedure. They are : (1) adsorption, a process of attracting and concentrating a dissolved substance by an adsorbant, in this case the filter paper. The strongly adsorbed compound concentrates nearer as compared to one which is held less firmly by the surface forces. Further addition of pure solvent to the column pushes down the adsorbed component still further, the loosely held one more than that which is held fast. (2) Ion-exchange acts by interaction of ions due to their polarities, in paper chromatography between ions of the mixture required to be resolved and the polar constituents of the cellulose and impurities present in the paper. Such a reaction effects the rate of spread of the various ingredients in the mixture used. (3) Partition between two immiscible phases is by far the most important feature which determines separation, when the two immiscible solvents containing the components to be separated are mixed together.

There are three important rules employed in the procedure of paper chromatography :— (1) The composition of the flowing solvent should be kept constant throughout the development. This is done by keeping the chromatogram in an enclosed chamber, the space of which is saturated with the developing solvent at contant temperature. (2) The developing solvent should move at a relatively slow rate 2-3 cm. per hour. This depends on paper used, the size of the "wick," composition of solvent, and the temperature of the chromatogram chamber. (3) Solvent used should be such that the components to be separated have a small but definite solubility in it.

The methods used are several, but the one's most popular are, (1) Ascending Chromatography. In this method paper strips are used. These strips are contained

in an air-tight chamber of suitable size. The ends of these strips hang in a container with a solvent placed at the bottom of the chamber. (2) Circular paper chromatography. This method has the following advantages, and has been made use of in the present investigation :— (1) The simplicity of the apparatus, (ii) the speed with which the chromatogram can be run, (iii) reproducibility and (iv) sharpness of separation.

This method has been employed to study the amino acid content of normal and abnormal C.S.F. in the department of Neurology, Christian Medical College Hospital, Vellore. In this paper the study of normal C.S.F. alone is described and standardization of technique developed such as to make it possible to assess abnormalities in the amino acid content of the fluid in disease.

Experimental Technique :

The apparatus :—

(i) Separatory funnel, about 300 cc. capacity.

(ii) Petri dishes, three in number, sizes used by us were 5½", 4" and 6" diam. respectively.

(iii) One micropipette.

(iv) Half a dozen Wasserman test tubes and two Centrifugal tubes.

(v) One 10 cc. caliberated pipette and a few pipettes with rubber teats.

(vi) Wooden rack for test tubes.

(vii) Whatman paper No. 1.

(viii) One plastic disc, 5" diam. with a central small hole to cut the disc of the paper to a uniform size which fits in the petri dishes. The hole in the plastic disc gives the centre of the paper disc.

(ix) A pair of scissors.

(x) A beaker to support the paper when it is being used to spot the extract for investigation.

(xi) Blower giving warm air for drying the paper.

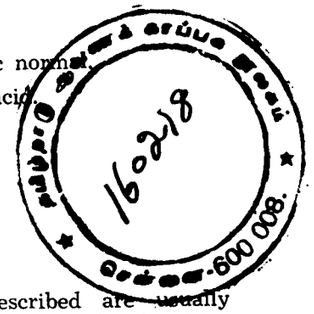
(xii) Viewing box, preferably x-ray viewing box — if available.

(xiii) A vacuum dessicator.

(xiv) 100 cc. cylinder.

(xv) A few 500 cc. glass beakers.

(xvi) A few glass bottles for keeping reagents.



The reagents :—

(i) Alcohol Butylic normal.

(ii) Glacial acetic acid.

(iii) Ninhydrin.

(iv) Acetone.

(v) Pyridine.

(vi) 90% alcohol.

(vii) Chloroform.

All of the items described are usually available in any clinical or biochemistry laboratory and hence the procedure is both simple and easy for its use. The reagents are prepared as follows :—

1. The solvents :—

(i) Alcohol Butylic normal.	40 cc.
Glacial Acetic Acid.	10 cc.
Distilled water.	50 cc.

are measured in the calibrated cylinder and mixed and shaken in the separatory funnel. The mixture will, in a few minutes, separate into two layers. The lower portion is drained off and the upper part is used as solvent.

(ii) Pyridine water is prepared by mixing pyridine with water in the ratio of pyridine 8 and distilled water 2.

2. Spraying reagent is made by making 0.1% acetone solution of Ninhydrin.

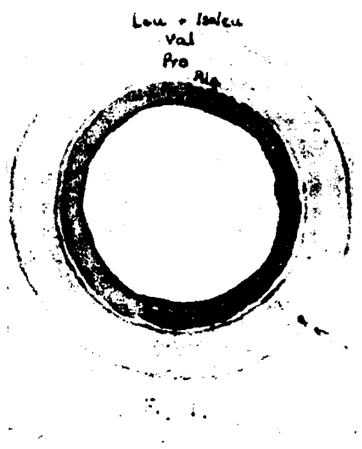
The Experimental Protocol :

About 2 cc. of blood-free cerebrospinal fluid is collected from the patient. After using various volumes of C.S.F. varying from ½ cc. to 3 cc. we have finally concluded that 1½ cc. of fluid gives uniformly good results. To this fluid 1½ cc. of 90% alcohol

is added. A very fine precipitate forms. This may need centrifuging for about 10 minutes at about 2,000 revolutions per minute for cleaning the mixture. To this precipitate free mixture 3 cc. chloroform (anaesthetic) is added and shaken. A thick white cloudy mixture forms. This is kept in a test tube. In about half an hour to one hour, a clear supernatant fluid comes at the top. This is usually about 1.8 cc. It is pipetted off and 0.6 cc. of it is taken for spotting. Spotting is done with a micro-pipette and enough extract is dropped at a time to give a spot of about ½ to ¾ cm. diam. Each drop must dry before the next one is put on. This procedure sometimes takes two to three hours to finish it at room temperature. The extract could be concentrated by dessication, but this increases the salt concentration in the extract also which interferes with proper partition of the amino acids.

The petri dishes are so arranged that 5½" diam. dish contains the one which is 4" in diam. and both are covered over by the 6" diam. petri dish. The bottom of 5½" diam. dish is covered with a disc of filter paper soaked in water, over this 4" diam. petri dish rests. This water keeps a proper amount of water vapour pressure in the petri dish chamber and allows spread without interference caused by drying of the paper if the chamber did not have moisture enough. The 4" diam. petri dish contains about 10 to 15 cc. of solvent No. 1 which we have used most frequently or any of the other solvents as and when needed.

The Whatman paper No. 1 disc is already cut to the size of the petri dishes by means of the plastic disc which also gives the centre for the paper disc. It is spotted with the extract, is perforated in the centre to accommodate a wick made by rolling a small strip (2 mm. x 2 cm.) of the Whatman paper No. 1 and is supported on the 4" diam. petri dish with the wick dipping into the solvent. All this is covered over with 6" diam. petri dish to make an air-tight chamber in which the vapour pressure of the solvent and water are kept uniform automatically. A proper vapour pressure is very important for spreading. The size of the wick is determined by repeating the experiment. The aim is to get the rate of spread about 2 to 4 cms. per hour. The spreading continues for a couple of hours till it reaches just a few mm. short of the circumference of the paper disc. At this stage the disc is removed with the wick sticking in it. It is dried and again put in the chamber for a second spread up to the margin of the first one. It is repeated four times. Giri et al has given this procedure the name, multiple development technique. At the end the disc is finally taken out with the wick removed, and allowed to dry and then soaked with 0.1% Ninhydrin in acetone. Drying is done by means of the blower and then the disc is left in the dessicator. Beginning about 15 minutes after spraying with Ninhydrin, the coloured circles of amino acids develop to full intensity in about two hours time. At this stage the disc is taken and viewed on the viewing box. The following bands are easily seen and distinguished. From without inwards they are : — (1) A purple band about 1 mm. in width. (2) A faint purple band about 2 mm. in width. (3) A yellow band about 2 to 3 mm. in width. (4) A bright purple band about 2 mm. in width. (5) A thick,



bright band about 4 to 5 mm. in width. These five bands have been identified to be those of Leucine-Isoleucine, Valine, Proline, Alanin and Glutamic Acid + Aspartic acid, respectively in that order, from periphery to the centre (Fig. 1). (Giri, personal communication).

Almost every normal C.S.F. specimen gives these bands, only the yellow proline band varies considerably in size and intensity in various specimens. Our standard for normal C.S.F. specimen is that it contains chlorides, proteins and sugar within normal range, is not blood tinged, has no increased cells and does not give positive Kahn or colloidal mastic reaction. The total number of such specimens examined are 25 in all.

Comment :

In two specimens out of twenty five a faint light-blue evanescent band could be detected just outside proline (yellow) band. This is suspected to be that of Tryptophane and Tyrosine. This band has not yet been properly identified. Its situation, however, fits in with Tryptophane and Tyrosine. Usually when the Glutamic acid + aspartic acid band is very thick, dense and bright, proline band is extraordinarily faint.

Discussion :

The importance of carbohydrates in the metabolic process of neural tissue has been well-established. The significance of proteins for the function of this tissue has been emphasised only recently. Amino acid study of neurone has been delayed because of the idea that neurone is not a cell which can grow or multiply hence proteins would play only an insignificant role in its activity as compared to a constituent like Glucose which gives energy more easily. The subject of proteins, however, has been brought into prominence by the studies of Casperson[1] and Hyden[11] who emphasised that during fatigue of the neurone, it is the amino acid metabolism of this cell which is significantly effected. It has been further commented that since nerve tissue is ectodermal in origin neurokeratin is present in this tissue in great predominance. Neurokeratin is the chief protein present in the nerve tissue. Yet, the neurone, as Monne[16] remarks, owes its functional capacity to its efficiency, (due to its nucleo-proteins), to form by chemical combination of amino acids unstable globulins which give rise to such elusive phenomena as memory, conception, or imagination. Each new idea or experience might be stored in a particular aggregates of amino acids forming protein molecules. Instinct would be interpreted in such a scheme as the inheritance of specific amino acid constellations adapted to prepare certain pre-established proteins from generation to generation.

The amino acids in the C.S.F. are only a reflection of those present in the nerve tissue. Solomen[21] et al have reported values for the total amino acid nitrogen and for eleven individual amino acids in the C.S.F. The estimations were carried out by microbiological methods and varied from ¼ to 1/15 of the amounts of amino acids present in blood. They, however, emphasised that in the C.S.F. Glutamine and Glutamic acid content is comparatively very high as compared to other amino acids. Their quantity is about 40 to 80% of the total amino carboxyl-N of brain. This has been confirmed by Richter[19], Dawson[8] and Rees[18] both in normal as well as in psychotic patients. It has, moreover, been demonstrated that brain slices are capable of taking up Glutamic acid from the suspension medium against a concentration gradient if glucose is present in addition. Microbiological methods however, are not easily available for clinical investigation and hence paper chromatography holds great promise in this field. Even though this method may not give all the details, yet its findings are likely to be more widely applicable from the point of view of clinical assessment.

We have demonstrated by the method standardized in this paper, that whilst five and occasionally seven amino acids, e.g. Leucine, Isoleucine, Valine, Tyrosine,

Tryptophane, Proline, Alanin and Glutamic Acid + Aspartic Acid are detectable, Glutamic Acid + Aspartic Acid alone appear as the most prominent constituent. This, at first sight, appears to be a peculiar feature when we understand that Glutamic acid given intravenously does not increase the Glutamic acid content of the neurone or C.S.F. This may either mean that the amino acid is not able to pass the blood brain barrier or is so readily metabolised when in excess that the increased quantity cannot be demonstrated as such. Glutamine, an ammonia compound of Glutamic acid, however, passes the blood brain barrier more easily and hence either this compound or prolin, which also can be converted to Glutamic caid, are the sources of this amino acid in the nerve tissue. A definite quantitative relationship of Glutamic acid band to prolin can be demonstrated in the chromatogram.

There are three principal reactions of glutamic acid described biochemically, i.e. (1) Deamination, (2) Transamination and (3) Amidation. These three reactions are ultimately integrated into a single system whose main function is deionization and removal of intracellular ammonia[22]. Ammonia is known to be produced in all neurone activity and if in excess it produces irritability by liberating acetylecholine to the extent producing convulsions. Some of the most powerful drugs acting on the nervous system are compounds containing Quarternary Ammonium group.

The other functions of Glutamic Acid are that it may serve as a substitute for the respiration of brain, but only when glucose utilization or supply fails. It helps in acetylecholine synthesis by being an essential constituent for the formation of coenzyme A which must be present in adequate quantity before choline acetylase can act to form acetylecholine. This in normal brains seems to have a very limited value. It has been also recently described that the presence of Glutamic Acid in the neurone effects its permeability so that potassium ions are retained inside the cell. This happens especially after a stimulus which depletes this ion due to pumping out. The effect of Glutamic Acid as an adrenergic agent has been established already. The hyperglycaemia demonstrated when Glutamic Acid has been injected is due to the fact that it stimulates excessive production of Adrenalin. It has been found of late that in some cases of Grandmal, Glutamic Acid when given in big doses enhances discharge of abnormal waves as seen in E.E.G.

The significance of other amino acids, e.g. Leucine, Isoleucine, Valine, and Alanin for the brain tissues specifically is not known. They, however, are essential amino acids, and, therefore, are necessary for the formation of specific cell proteins which maintain the life and activity of the cell.

Summary :

Apparatus and technique for amino-acid analysis of C.S.F. has been described. The method used has been that of multiple development. 25 normal C.S.F. specimen have been analysed. It is pointed out that these specimens usually show five bands. Beginning from periphery to centre they indicate Leucine, Isoleucine, Valine, Proline, Alanin and Glutamic Acid + Aspartic Acid. Glutamic acid + aspartic acid band is the most prominent. Proline band although constant yet varies considerably in intensity of colour and width — Tyrosine and Tryptophane bands are found infrequently and are evanescent.

It has been pointed out that 1½ cc. C.S.F. extracted with 90% alcohol and Chloroform gives the best result. 0.6 cc. of the extract is spotted for analysis. The functional significance of amino acids demonstrated by Chromatography has been discussed and it has been described that the main utility of Glutamic acid is to neutralise Ammonia which is a well-known irritant for the neurone. Other amino acids are essential constituents required to maintain the life and activity of the cell.

It is quite clear that knowledge of amino-acid content of C.S.F. has considerable importance. Paper chromatography gives an indication as to what amino acids can be demonstrated in this fluid in the normal and abnormal state. The method can be developed further to make it quantitative roughly by noting the intensity and width of the various bands and more accurately by chemical tests.

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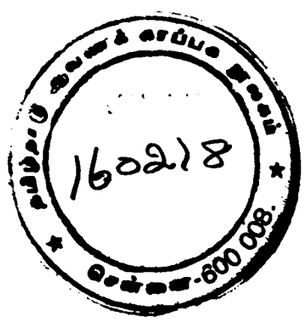

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