FURTHER STUDIES ON THE MODIFIED TAKATA REACTION (M.T.R.) ON THE CEREBROSPINAL FLUIDS OF NEUROLOGICAL AND PSYCHIATRIC PATIENTS.

By H. H. FLEISCHHACKER, M.D.

From the Pathological Laboratory, Shenley Hospital (Middlesex County Council), Shenley, Herts.*

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PART I.—COMPARISON BETWEEN THE M.T.R. PLOTTED AS A "TIME-CURVE" AND THE LANGE TEST.

Ucko (1935) showed that the reaction time of the Takata test on serum can be reduced from 24 hours to 90 minutes by modifying the concentration and volume of the reagents.† These reagents were applied with a further slight modification in the amount of the fluid tested and as a one-tube method for cerebrospinal fluid (Fleischhacker, 1938). 177 cerebrospinal fluids were examined, and the conclusion was drawn that the M.T.R. will indicate even very fine disturbances in the protein composition of cerebrospinal fluid. Later the test, together with others, was applied to another 165 C.S.Fs. from neurological and psychiatric patients and was found to give weak positive results in many psychoses of the so-called "non-organic" type (especially typical schizophrenics), while it remained negative in manic depressives and neurotic disorders (Fleischhacker, 1943).

Although attention had been drawn to the importance of the time factor in the development of the reaction in the previous papers, no attempt had been made then to plot the development of the turbidity as a time curve and to compare this with the Lange test.

A systematic study on this subject has now been made, and the results obtained are described in the first part of this paper.

MATERIAL, TECHNIQUE AND RESULTS.

The material used in this section consists of 122 cases, most of them of "organic" origin, i.e. of active neurological processes.

Besides the M.T.R. and Lange test, total protein, Nonne and Pandy, cell count and Wassermann and Meinicke estimations and tests have been made. With the exception of Wassermann and Meinicke, all tests have been carried out on fluids not more than two hours old.

Techniques.

(a) *Cell Count.*

A Fuchs-Rosenthal counting chamber has been used and 3 c.mm. have been counted. The results have been expressed as Cells/3. The cells were always given sufficient time in which to stain thoroughly and to settle down in the chamber.

(b) *Nonne.*

The Ross-Jones modification (ring test) was used and readings were made after 3 minutes. The reaction was made by allowing a volume of saturated

* Paper read at the South-Eastern Divisional Meeting of the R.M.P.A. at Shenley Hospital on October 10, 1944.

† This serum test is now known as the "Ucko Test" (for details see Ucko [1942]).

ammonium sulphate solution to run through an equal volume of C.S.F. and to collect as a layer beneath the C.S.F.

(c) *Pandy.*

One drop of C.S.F. was added to 1 c.c. of 10 per cent. aqueous phenol placed in a small tube. The tube was shaken after 10 seconds and the readings made. The tube was kept for a further 1 hour for other purposes.

(d) *Total protein.*

The turbidometric method of precipitating the proteins with salicyl-sulphonic acid and comparing with a set of standards was used. The error possible in this rough method is usually accepted as ± 3–4 mgm. per 100 c.c. in the lower concentrations.

(e) *Lange.*

A commercial aurosol has been used. Its sensitivity has been checked by controls with sodium chloride solutions of different strengths and by the results obtained with fluids of known diagnosis. It was also checked from time to time with " home-made " sols. The C.S.F. dilutions ranged from 1 : 10 to 1 : 5120 in serial dilutions. The control tube was compared with each individual tube of the test and the individual tubes were compared with each other. All comparisons were made against a white background.

If the Lange test is read in the manner described, weak positive results, i.e., small changes in colour, are not overlooked and recorded as " Negative " or " Normal."

Reading was made after 24 hours at room temperature (14–18° C.). If the tubes are immersed in a water bath at 37° C. a reading can be made after 2 hours, or by using an ice-cooled sol readings can be made after 2 hours at room temperature. Under these circumstances the sol is rendered more sensitive, and the readings obtained cannot be compared without modification with those published here.

The results of the Lange are expressed as follows :

0. Red (practically the same as the control tube).
1. Red-Mauve.
2. Mauve.
3. Mauve-Blue.
4. Blue.
5. Sedimentation (colourless).

Colour changes existing between those mentioned are expressed as $x + \frac{1}{2}$, i.e., " nearly mauve " = $1\frac{1}{2}$, " nearly blue " = $3\frac{1}{2}$.

In practice, such reading renders a still finer degree of evaluation. Lange, in a recent paper (1939), has given instructions for a still more " quantitative " evaluation of the test.

(f) *Modified Takata Reaction* (*M.T.R.*).

A detailed description of this test has been given in previous papers (Fleischhacker, 1938, 1943, and Lane, 1945). It has been pointed out previously that the reaction time of the test is influenced by the temperature of the environment. This is due partly to the increasing alkalinity of the reagent-fluid mixture by the liberation of carbon dioxide from the fluid. This is known to influence the Weichbrodt reaction (Fleischhacker, 1931), and Lane (1945) has shown that this holds good for the M.T.R. The liberation of carbon dioxide may, however, not be the only factor responsible for the decrease in reaction time, for the overlaying of the mixture with liquid paraffin will not prevent a speeding up of the reaction at temperatures above 25° C. As we allow the reaction to continue for 90 minutes, the practice of overlaying the mixture with liquid paraffin is recommended, as this prevents false reactions given late and which can be influenced by the change in pH. The overlaying by liquid paraffin makes the use of a water bath unnecessary at room temperatures up to about 20° C.

The occurrence and increase of very faint turbidities can be easily marked and followed up with a little practice. That naked-eye observation is sufficient could

be shown by the results obtained by using the Hilger photo-electrometer as a nephelometer.

In the routine examinations we find it most convenient to hold the tube against a dark background and the following definitions are given :

1. ± (Doubtful.)
2. V.F.T. (Very faint trace.) Turbidity just visible.
3. F.T. (Faint trace.) Turbidity plainly visible against a dark background.
4. T. (Trace.) Turbidity visible without dark background.
5. W+ (Weak positive.)
6. + (Positive.)
7. ++ (Strong positive.) (Frequently with sedimentation.)

In addition to the above definitions we are accustomed to make use of a finer definition, and for this reason the signs (*a*) > and (*b*) < are used to signify (*a*) " more than " and (*b*) " less than."

It has to be emphasized that the conditions laid down for the performance of the M.T.R. must be strictly observed, otherwise false results will be obtained. If the test is carried out under liquid paraffin in chemically clean test tubes and using fresh C.S.F. in a moderate room temperature, the results will be found to be consistent and reliable. It is sometimes difficult to evaluate correctly a reading above a " trace," and a reading above a " trace " may be called " weak positive " when in actual fact it is not quite as strong as " weak positive." False results have been given by fluids containing apparently high concentrations of barbiturates, and the presence of traces of antiseptic in which the lumbar puncture needles are kept is likely to give false turbidities, but the turbidity resulting from the presence of contaminants is more " earthy " than the true M.T.R. turbidity, and usually commences to sediment within 20 minutes, although the opacity is weak.

Readings of the M.T.R. are made at intervals of 0, 1, 3, 5, 8, 10, 15, 20, 30, 60 and 90 minutes, and the result is plotted in graph form in a similar fashion to the reading of a Lange.

Comparison between M.T.R. and Lange.

In order to make the two tests comparable, the following scheme has been adopted :

Lange. Tube.	Lange. Dilution.	M.T.R. Minutes.	M.T.R.
1	1 : 10	0	1
2	1 : 20	3	2
3	1 : 40	5	3
4	1 : 80	8	4
5	1 : 160	10	5
6	1 : 320	15	6
7	1 : 640	20	7
8	1 : 1280	30	8
9	1 : 2560	60	9
10	1 : 5120	90	10

Intensity of Reaction.

Lange.		M.T.R.
Red	0	Clear.
Red-mauve	1	Faint trace (F.T.).
Mauve	2	Trace (T.).
Mauve-blue	3	Positive (+).
Blue	4	Strong positive (++).
Sedimentation	5	Strong positive with sedimentation.

This scheme is slightly different to the one adopted by Lane (1945), at a time when we were not so experienced. The scheme adopted here does more justice to the lesser intensities of the reactions, while a somewhat greater discrepancy exists with the stronger intensities. The Strong Positive (++) or " 4 " of the M.T.R. is obviously stronger than the Blue (4) of the Lange Reaction. In a number of

cases a Lange reading of " 5 " will be given where the M.T.R. will not sediment within 90 minutes. Lane's scheme takes more account of these stronger reactions. The readings are entirely empirical, and it should be noted that the two reactions cannot be compared from a mathematical point of view.

Classification of the Curves Compared.

The usual Lange classification of " Paretic," " Tabetic," and " Meningitic " curves is misleading and only of a pseudo-diagnostic value. A " Meningitic " curve can be obtained in the absence of a meningitis, and indeed in the absence of any inflammatory condition. Similarly the two " syphilitic " types are encountered quite often without syphilis being present. To overcome this difficulty, some laboratories speak of " Left," " Middle," or " Right " depressed curves where Left = Paretic, Middle = Tabetic, and Right = Meningitic type. Lange (1939) differentiates 6 types according to the optimal (or maximal) depression of the reaction. These last-named classifications are not suitable for our present purpose, as the forms of the two reactions (Lange and M.T.R.) are different. The difficulty may be overcome to some extent by referring to those causes which for the time being are thought to have most influence on the curves—namely the Albumin-Globulin ratio of the C.S.F. We are well aware that other factors such as pH, quantitative and qualitative fractions of the proteins (euglobulins and others) may influence the development of the reaction, and that these factors may influence both reactions to a different degree. For all practical purposes, however, these exceptions may be overlooked for the time being, and the most probable factor, i.e., the Albumin-Globulin ratio, will be used as the basis for a classification.

The classification used here is therefore :

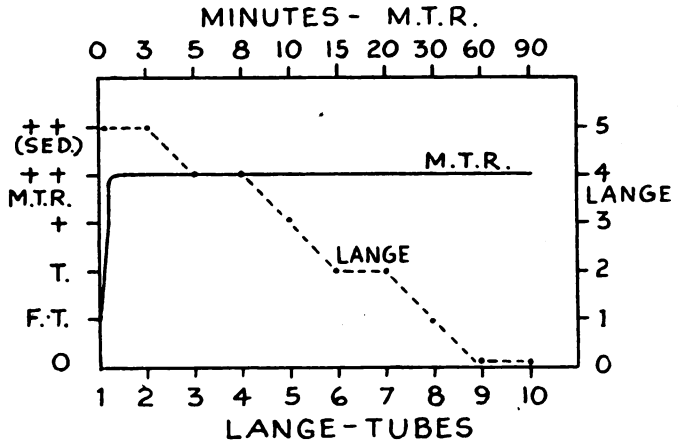
1. The " G " (globulin) type . . Paretic.
2. The "A" (albumin) type . . Meningitic.
3. The " GA" type . . . . Tabetic.

It will be appreciated that in a mixed neuropsychiatric material the typical types (G and A) will be rare, and the majority of curves will belong to a not always very well defined "GA" group.

Results.

1. " *G* " (*globulin*) *Type Curves.*

This type gives a Lange reading of approximately 5.5.5.5.4.3.2.1.1.0 and needs no explanation. The M.T.R. in such cases is characterized by an instantaneous Strong Positive which may or may not sediment. There were 8 cases of G.P.I. The results are recorded graphically as follows :



Case 226 (G.).—*Total protein:* 0·060 per cent. Nonne positive (++); Pandy positive (+). *Cells:* 85 lymphocytes, 13 polymorphs, 1 endothelioid, 12 red cells in 3 c.mm. *Wassermann:* Positive (+++). *Meinicke:* Positive. *Diagnosis:* G.P.I.

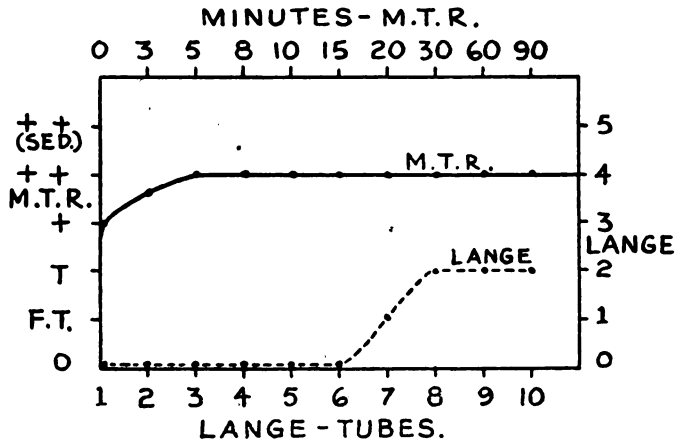
There was agreement between both curves in 7 of these cases. Two of them had a slight "A" admixture which could be well recognized in both tests. The eighth case was one of Juvenile (Congenital) G.P.I., and there was slight disagreement between the curves in that the M.T.R. gave a typical " G " curve while the Lange showed a slightly modified " G " curve with an "A" admixture. Clinically there was, as is so often in these cases, a mixture of paralytic and meningovascular symptoms, so that the Lange may have given a better expression of the pathological processes.

2. The "A" (Albumin) Type.

The "A" type curve is subdivided into two groups.

Sub-Group "A_1."

This was met in a few cases of severe meningitis (after mastoiditis, cerebral abscess, etc.), but also in non-meningitic disorders. There were 7 cases.



CASE 230 (A_1).—*Total protein:* 0·180 per cent. Nonne, positive (++); Pandy, positive (++). *Cells:* Pus cells +++. *Diagnosis:* Meningitis following mastoiditis. Bacteriologically there was a mixed infection.

The typical Lange curve is 0.0.0.1.2.3.4.4.5.5, while the M.T.R. corresponding to the Lange in these fluids is characterized by an instantaneous Weak positive turbidity which reaches a Strong positive in about 5 minutes. There was good agreement between the 2 curves in 5 of the 7 cases, while in 2 (not of inflammatory origin) the Lange reactions might have fitted better into the second sub-group of the "A" type.

Sub-Group "A_2."

Here the typical Lange curve is 1.1.2.2.3.3.4.4.3.3. The group consisted of 15 cases of quite different aetiology. While the M.T.R. is characterized by an instantaneous turbidity, less intense than in the "A_1" sub-group, it increases slowly throughout the greater part of the reaction time. The two reactions agreed in the following 13 cases:

2 cases of meningococcal meningitis.
3 cases of prolapsed intervertebral disc.
6 cases of neuro-syphilis.
1 case of polyneuritis.
1 case of " cerebellar disorder " (total protein 0·120 per cent., Nonne +, Pandy ++, cell count only slightly increased).

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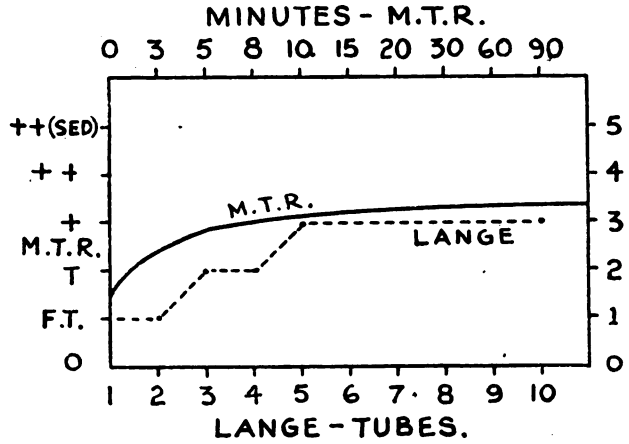
In two cases (post-vaccinial encephalitis and untreated trypanosomiasis) there was disagreement between the two reactions. The M.T.R. was more of the "A_2" type, while the Lange showed a modified " G " type curve. Incidentally, among the group of 30 cases described so far, these two cases were the only ones showing a noteworthy qualitative disagreement between Lange and M.T.R.



CASE 329 (Type A_2).—*Total protein:* 0·150 per cent. Nonne, +; Pandy, ++. *Cells:* 7,000 in 3 c.mm. *Diagnosis:* Meningococcus meningitis.

There were two small groups comprising 10 cases in all, in which in one group the Lange did not develop, and in the second where the M.T.R. did not develop correspondingly to the other routine reactions. It is interesting to note that in 3 of these last cases (? polyradiculitis or arachnoiditis, trypanosomiasis* and ? amyotrophic lateral sclerosis), Pandy's reaction was definitely stronger than the Nonne. A fourth case was a patient with a rapid generalized softening of the brain.

3. *The " GA" Type Curves.* (Tabetic.)

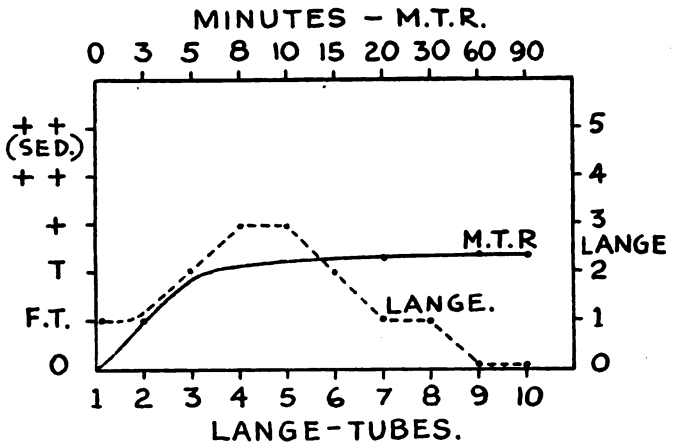
This type forms the main group of our material.† There are again two sub-groups, the larger group having a Lange which gives most change in the higher concentrations, and where the M.T.R. reaches its maximum within 10 or 15 minutes (sub-type Ga). In the second and smaller sub-group the Lange is more drawn out, and the maximum depression is seen in the lesser concentrations while the M.T.R. develops later (sub-type gA). Graphically, the peak of the curves lay between " 2 " and " 3," and consequent to the moderate strength of this reaction type, small errors in reading may produce relatively marked alterations in the forms of the curves. Therefore in a number of these cases, due to the factor mentioned above and also due in part to the relatively slight changes in the C.S.Fs. if compared with types " G " and "A," the picture is not always very outspoken.

Sub-Group " Ga."

The material in the group comprised 57 cases of mixed neurological (central and peripheral) cases and also a number of schizophrenics. There was good agreement in 51 cases (see Case 296), and still reasonably good agreement in the remaining 6 cases between the two reactions.

* This case has been published by Lt.-Col. Bomford, R.A.M.C. (1944).

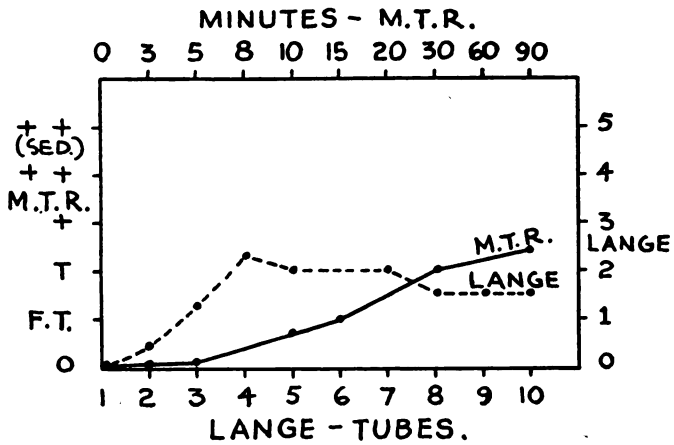
† The material published in Parts 2 and 3 of this paper also belongs more or less to it.



CASE 296 (Ga).—*Total protein:* 0·020 per cent. Nonne, faint trace; Pandy, doubtful. *Cells:* 1 lymphocyte, 13 red cells in 3 c.mm.

Sub-Group "gA."

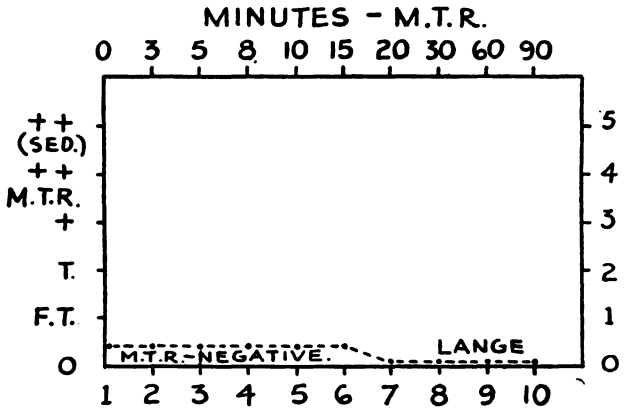
There were only 9 cases. There was good agreement between the two reactions in 6 cases and not quite so good an agreement in 2 cases. In the ninth case there was a definite qualitative disagreement between the two tests. The Lange was almost negative while the M.T.R. was definitely of the "gA" type (total protein 0·020 per cent.; Nonne doubtful; Pandy trace; cells, 1 lymphocyte in 3 c.mm. Diagnosis: Prolapsed intervertebral disc).



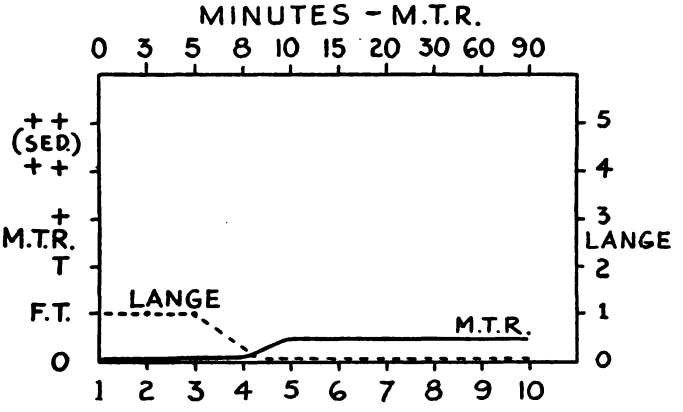
CASE 250 (gA).—*Total protein:* 0·020 per cent. Nonne, negative; Pandy, negative. *Cells:* 2 lymphocytes in 3 c.mm. *Diagnosis:* Schizophrenia.

There is a last group of 16 cases where the reactions were either negative or doubtful.

The ideal negative reaction is that where the curves do not show any appreciable change. But in some cases there were slight changes in the Lange of not more than "1" intensity in 4 (or 5) tubes, and a very faint trace in the M.T.R. not appearing before 10 or 15 minutes and not increasing in strength. These very slight changes might also be called negative. Both reactions agreed in 10 negative C.S.Fs. In 5 cases both reactions were so very little outspoken in type (qualitatively) and intensity (quantitatively) that they should be classed as doubtful. So there were 15 cases in which agreement was found.

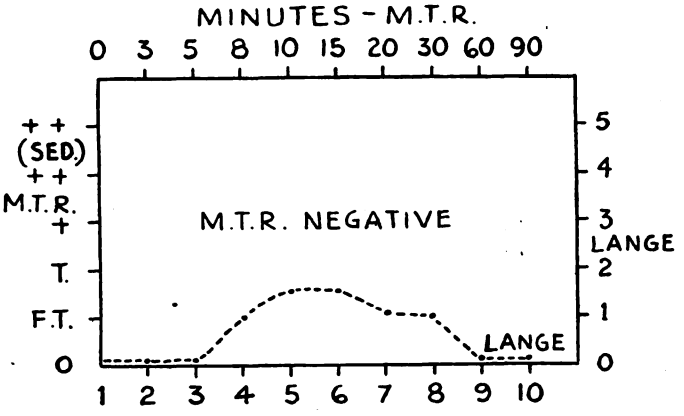


CASE 283 (Negative).—*Total protein:* 0·010 per cent. Nonne, negative; Pandy, negative. *Cells:* 4 lymphocytes in 3 c.mm. *Diagnosis:* Mental deficiency.



CASE 273 ("Still" negative).—*Total protein:* 0·015 per cent. Nonne, negative; Pandy, negative. *Cells:* 3 lymphocytes in 3 c.mm. History of syphilis two years previously.

In the last case of this group (Schilder's disease ?, tuberous sclerosis ??) there was a doubtful Lange test (0.0.0.1.1½.1½.1.1.0.0.) while all the other tests were definitely negative.



CASE 291 (Doubtful).—*Total protein:* 0·015 per cent. Nonne, negative; Pandy, negative. *Cells:* 2 lymphocytes in 3 c.mm.

CONCLUSION.

The plotting of the Lange and M.T.R. " Time-Curve " reactions are based on quite different principles. The Lange is a geometrical dilution, while the M.T.R. is a curve based on time and has no mathematical proportions. In spite of this both curves gave good corresponding results in about 80 per cent. of the cases and still reasonably good agreement in a further 10 per cent., leaving 10 per cent. in which an active disagreement is noted.

The analysis of over 120 cerebrospinal fluids, most of them of " organic " origin, shows that the M.T.R., similarly to the Lange, can exhibit three main types of curve when plotted as a " time-curve " reaction:

1. *Type " G."*—Corresponding to the so-called " Paretic " curve of the Lange reaction. It is characterized by a marked turbidity appearing at once, with or without sedimentation appearing within the 90 minutes of the reaction time.

2. *Type "A."*—This curve corresponds to the " Meningitic " Lange curve. Here the turbidity appears at once to a strength of " trace " or stronger, and increasing to its maximum either within a few minutes (sub-type A_1), or developing slowly throughout the greater part of the reaction time (subtype A_2).

3. *Type " GA."*—The curve corresponds to the " Tabetic " Lange curve. The characteristic feature is either a turbidity developing during the first 1–3 minutes and reaching its maximum within 10–15 minutes (sub-type Ga), or appearing later and increasing slowly in intensity (sub-type gA).

Although the intensities of the reactions of the " GA" type are usually much less marked than in types G and A, and consequently small errors in reading will have a comparatively strong effect on the form of the curves, it is noteworthy that the qualitative and quantitative agreement was on the whole satisfactory. The same holds good for the " doubtful " and " negative " curves.

The findings described here are in good principal agreement with those from the examination of well over 100 cerebrospinal fluids examined later and reported in the second and third parts of this paper.

PART II.—THE SIGNIFICANCE OF SMALL CHANGES IN THE REACTIONS.

In spite of the close agreement between the two reactions, it is not suggested that the M.T.R. should replace the Gold Sol Test. The M.T.R. has the advantage of being simple and being quickly performed. It uses only one tube, and its results (turbidities) are easier to read in doubtful cases than the slight colour changes of the Lange. It also has the advantage of giving a characteristic change within 30 minutes or less (although it should be followed up for 90 minutes) as against the 24 hours reaction time needed for the Lange.* The Lange has the advantage of giving somewhat more " characteristic " curves, of using a much lesser quantity of cerebrospinal fluid, and the number of tubes will show up possible technical errors more easily than the one tube used for the M.T.R.

If one is content to obtain " characteristic " curves in " characteristic " diseases the Lange test may be preferred, although in most of such cases the " diagnosis " from the cerebrospinal fluid can be made without using a colloidal test at all. But if one thinks differently and wishes to use the colloidal reactions in difficult clinical cases, especially in order to describe the presence or absence of " organic " components, then both reactions should be used in a more " quantitative " way. The difficulty in the evaluation of such borderline cases is generally that very often some of the tests (total protein, cells and Pandy) seem to be negative against a " doubtful " Nonne and a similar Lange or M.T.R., and then the cerebrospinal fluid is usually given as " still normal." The careful use and reading of the two colloidal reactions, satisfactorily agreeing and therefore supporting each other. will make it easier to make a decision, and it will be seen that in such circumstances quite a number of cerebrospinal fluids will turn out to be slightly pathological.

* If the water bath (37° C.) technique or an ice-cooled gold sol are used for the Lange, the reaction time is considerably reduced. One has to bear in mind, however, that by the speeding up of the test the sensitivity of the sol is considerably increased, and the changes even in " normal " curves are greater than those described in this paper.

It is not very easy to define a normal cerebrospinal fluid. One might prefer to answer the question indirectly by saying that a normal cerebrospinal fluid is a fluid which we do not receive for examination as a rule. A lumbar puncture is not done unless a patient is considered to be suffering from an organic disease of the central nervous system (or otherwise). The great majority of fluids described in the first part of this paper have been drawn from patients suffering from an active organic neurological disorder* (in order to establish that the results obtained were of pathological significance), and also the fluids received from psychotic patients are usually from those of the " organic " type. But among more than 450 cerebrospinal fluids examined in this laboratory during the last two years, there were well over 20 which might be considered as not being pathological. They came from neurotic and manic-depressive patients, from a few cases of suspected, but not confirmed neurological disorders, and from patients clinically and serologically cured of syphilis.

On the experience of this small material, if compared with the volume of pathological fluids, we have come to the following conclusion with regard to the evaluation of the individual routine tests applied to cerebrospinal fluids. It will be seen that they are in agreement with the views expressed by Lange and Neel and their schools, although our results are obtained with less elaborate methods than those used by these authors, and also by Kafka and Samson (1928) and their followers. We think that a cerebrospinal fluid should not be considered as normal unless Pandy and Nonne are negative, total protein under 25 mgm. per 100 c.c., cells less than 5 in 3 c.mm., and Lange and M.T.R. negative, or if only *one* of these reactions shows a *very* slight deviation from these standards.

Evaluation of Results.

Pandy and Nonne Tests.

The tables show that there are a number of C.S.F. in which the Pandy and Nonne tests are negative, but which have slightly positive M.T.Rs. and Langes. This is a consequence of too early a reading being made of the tests. The agreement is much better if longer reaction times are used. It is suggested that neither of these two reactions should be read before 15 minutes have elapsed. We have, however, published the results obtained after 10 seconds for the Pandy and 3 minutes for the Nonne in order to show that even then a careful reading of the M.T.R. and Lange together will reveal possible abnormalities of the fluids.

Total Protein.

As the normal protein content of the cerebrospinal fluid is most likely not much higher than 20 mgm. per 100 c.c., readings of 25 mgm. per 100 c.c. should be regarded as possibly abnormal, indicative of a qualitative or quantitative change in the protein composition of the fluid.

Looking through the whole of our material with total protein readings of 25 and 30 mgm. per 100 c.c. there is only one case (338) where, apart from pathological Lange and/or M.T.R., no other change could be detected. For more material see Neel (1939) and Lane.

We think that in spite of the errors attached to the technique, values of 25 mgm. per 100 c.c., or higher, obtained by the turbidometric estimation of protein, will be of use in deciding that a " doubtful " fluid is really pathological.

Cell Count.

According to Neel (1939) a normal cerebrospinal fluid should not contain more than one cell in 3 c.mm. This is apparently confirmed in the greater number of our normal cases. Still, one might feel that this is a little rigid, and prefer Lange's (1939) formula that a normal cerebrospinal fluid does not contain more than one cell per c.mm. and probably less. Cell counts of 4/3 or 5/3 should be considered as " borderline," and anything higher as definitely abnormal.

* Most of these fluids were kindly given to us by Major T. Crawford, R.A.M.C., and I wish to express my thanks to him.

Modified Takata Reaction.

This reaction is definitely negative when the reagent-fluid mixture remains clear. A " very faint trace " of turbidity occurring after 10 or 15 minutes and not increasing during the reaction time will also for the time being be considered as negative. " Trace " reactions are always pathological if the technique is properly carried out.

Lange Reaction.

This reaction is usually considered to be still normal if the colour change is not more than " 1," and even a " 2 " reaction in one or two tubes is often evaluated as non-pathological. The technique of performing and reading the test as described above and applied to active organic conditions demonstrates the close comparison with the other reactions, especially the M.T.R. It has taught us that a reaction of " 1 " in more than 5 tubes (perhaps even in more than 4 tubes) cannot be considered as normal.* Reactions with a change of " 1½ " in more than 2 tubes should be regarded as doubtful, and a " 2 " change even in 1 tube is of slight but definite pathological significance.

After having discussed the criteria of the individual tests, we publish now the findings on 56 fluids obtained from patients suffering from organic central nervous system disease (Tables I, II and III). The results are not those published previously by myself or Lane, and therefore support our former findings and deductions. They also support the views held by authors such as Lange and Neel.

Definitely negative cerebrospinal fluids, i.e., with cells below 5/3, Pandy, Nonne and M.T.R. all negative, Lange showing no change and total protein lower than 25 mgm. per 100 c.c. have been omitted, and also findings which would be generally considered to give the results of the examination as slightly pathological or abnormal.

The idea is, as mentioned above, to demonstrate how a careful evaluation and comparison of the routine tests will enable us in many cases, although not in all, to make a decision, although some of the results obtained are seemingly conflicting. It should not be forgotten that although cell count and protein reactions often run parallel, this need not always be the case. One sometimes finds as an expression of the slightest " mechanical irritation " of the meninges a slight rise in the cell count without a distinctive disturbance of the proteins, or, more often, and as most marked in the Froin syndrome, a strong increase of the proteins without an increase in the cell count.

The tables have been arranged in three parts according to the cell count. The first group gives the results of cases with a cell count of 11–15/3. This group is the smallest as, naturally, the proteins very often show a distinctive disturbance in several of the tests and such fluids have been omitted. The second group is composed of the findings with fluids having a cell count of 5–10/3, with finally the group with cell counts up to 5/3, the largest groups of this material.

In the following tables the Lange is given in figures, the M.T.R. is given at its 30 minute value, and in this, as with Nonne and Pandy, the strength of the turbidity is expressed as ± = doubtful, V.F.T. = very faint trace, F.T. = faint trace, T. = trace, W+ = weak positive, + = positive, ++ = strong positive. The prefix " L " found in some of the M.T.R. results stands for " Late " in such cases where the turbidity did not develop within the first ten minutes.

Other abbreviations used are: *Pro.*, Total protein in mgm. per 100 c.c. *N.*, Nonne. *P.*, Pandy. *M.T.R.*, Modified Takata Reaction. <, Less than . . . >, More than . . .

TABLE I.—*Fluids with a Cell Count of* 11–15 *Cells in* 3 *c.mm.*

No.	Cells.	Pro.	N.	P.	M.T.R.	Lange.	Diagnosis.
310	11/3	25	±	—	>Tr.	1.1.1.2.2.2.1.0.0.0	Spinal process C5.
332	11/3	30	±	±	>Tr.	1.1.1½.2.1½.1.1.½.½.0.	Organic cerebellar disorder.
270	12/3	30	Tr.	Tr.	W+	1.1.2.2.3.2.1.0.0.	Trypanosomiasis.
448	13/3	20	—	—	<Tr.	1.1.1.1½.1.1.1.1.0.0.	? Neurosyphilis.
439	14/3	20	L.F.T.	V.F.T.	>Tr.	½.½.1.1½.1½.1.½.½.0.0.	Epilepsy.
381	14/3	30	F.T.	F.T.	>Tr.	0.1.1.2.2.2.1.1.0.0.	Cysticercus.

* We wish to point out again that this does not hold good for the results obtained with the speeding-up techniques which render the sol more sensitive.

TABLE II.—*Fluids with a Cell Count of 5–10 Cells in 3 c.mm.*

No.	Cells.	Pro.	N.	P.	M.T.R.	Lange.	Diagnosis.
338	5/3	25	—	—	L.V.F.T.	½.½.½.½.0.0.0.0.0.0.	Jacksonian epilepsy.
264	5/3	20	±	F.T.	F.T.	½.½.½.0.0.0.0.0.0.0.	Traumatic instability.
428	5/3	23	±	—	±	½.½.1½.1½.1½.½.0.0.0.0.	Involutional melancholia.
358	5/3	20	±	—	Tr.	0.0.1.1.1.1.0.0.0.0.	Gumma of nose.
277	5/3	20	F.T.	—	Tr.	½.1.1.2.1.½.0.0.0.0.	Fainting spells; ? neurosyphilis.
451	6/3	20	—	—	—	½.1.1.1.1.1.0.0.0.0.	Spinal process ?
275	6/3	20	—	±	—	½.½.½.½.½.0.0.0.0.0.	Epilepsy.
317	6/3	20	—	±	L.T.	1.1.1.1.1½.0.0.0.0.0.	,,
379	6/3	20	—	±	Tr.	0.0.1.1.1.2.2.1.1.0.	Fainting attacks.
370	6/3	20	F.T.	±	Tr.	0.0.1.2.2.2.2.2.1.0.	Cysticercosis.
269	6/3	20	F.T.	F.T.	Tr.	1.1.1.2.2.2.1.1.0.0.	Wasting of left leg.
370	7/3	20	—	—	Tr.	0.0.½.1.1.1.1.1.0.0.	Epilepsy.
468	7/3	20	—	±	<L.Tr.	Not done	Jacksonian epilepsy.
410	7/3	20	±	—	<Tr.	0.0.0.1.1.1.1.0.0.0.	Old meningitis, now epileptic fits.
422	7/3	30	Tr.	±	VW+	1½.1½.1.½.0.0.0.0.0.0.	Encephalitis.
319	8/3	<20.	±	±	<Tr.	0.0.0.1.1.0.0.0.0.0.	Treated neurosyphilis.
299	8/3	30	±	±	>Tr.	0.0.0.0.1.1.1.1.1.1.	Organic psychosis.
387	8/3	25	±	±	>Tr.	0.0.½.½.1.1.1.½.½.½.	Organic neurological disorder.
426	8/3	25	F.T.	±	VW+	1.1½.2.2½.1½.1½.0.0.0.0.	Psychosis after head injury.
287	8/3	30	F.T.	F.T.	>Tr.	0.1.1.1.1.1.0.0.0.0.	Attacks of unconsciousness.
292	8/3	25	F.T.	F.T.	Tr.	1.1.2.2.1.1.1.0.0.0.	Sciatic neuritis.
325	9/3	25	—	—	±	½.½.1.1½.1½.1.½.½.½.0.	Unequal pupils.
388	9/3	25	±	—	Tr.	0.½.½.1.1.1.½.½.0.0.	? Cerebral tumour.
395	9/3	30	F.T.	F.T.	+	½.1.1½.2.2½.2½.2.1½.½.½.	Incipient G.P.I.
307	9/3	25	±	—	Tr.	0.0.1.1.1.1.1.0.0.0.	Optic atrophy.

TABLE III.—*Fluids with a Cell Count of 0–4 Cells in 3 c.mm.*

No.	Cells.	Pro.	N.	P.	M.T.R.	Lange.	Diagnosis.
334	0/3	20	±	—	Tr.	½.½.1.1.½.½.½.½.0.0.	Neurosyphilis.
328	0/3	20	±	—	>Tr.	1.1.½.½.½.½.½.0.0.0.	Amyotrophic lateral sclerosis.
311	0/3	30	—	±	±	0.0.1.2.2.2.1.0.0.0.	Cerebral tumour.
339	0/3	25	F.T.	—	Tr.	½.½.½.1½.½.½.0.0.0.0.	Organic dementia.
376	0/3	30	F.T.	±	W+	1.1.1.2.1.1.0.0.0.0.	Sciatic neuritis.
300	0/3	30	V.F.T.	F.T.	Tr.	½.1.1.1½.2.1½.1½.1.½.½.	Incipient neurosyphilis.
452	1/3	20	—	—	Tr.	½.½.1½.1½.1.1.½.½.0.0.	Gumma of skull.
337	1/3	20	±	±	Tr.	1.1.1.1.½.0.0.0.0.0.	Epilepsy.
295	1/3	25	±	±	V.F.T.	1.2.2.2½.2½.2.1.0.0.0.	? Neurosyphilis.
296	1/3	20	F.T.	±	W+	1.1.2.3.3.2.1.1.0.0.	Neurosyphilis.
439	1/3	25	F.T.	F.T.	Tr.	½.1.1.1½.2.1½.1½.1.½.½.	Incipient neurosyphilis.
265	1/3	20	±	Tr.	Tr.	½.½.½.½.0.0.0.0.0.0.	Prolapsed intervertebral disc.
291	2/3	15	—	—	—	0.0.0.1.1½.1½.1.0.0.0.	? Schilder's disease.
412	2/3	20	—	—	<Tr.	0.0.0.1.1.1.1.0.0.0.	Spinal lesion.
274	2/3	20	±	±	Tr.	1.1.1.1.2.2.1.1.1.1.	Neurosyphilis.
304	2/3	25	±	V.F.T.	Tr.	1.1.1.1.1.1.0.0.0.0.	Epilepsy with reflex disturbances.
323	2/3	30	±	±	Tr.	0.0.1.1.2.2.1½.½.0.0.	Spinal process.
385	2/3	25	±	F.T.	W+	½.1.1½.2½.2½.1.½.½.0.0.	,, ,,
336	2/3	25	F.T.	F.T.	W+	1.1.1½.1½.1.1.1.0.0.0.	Rapid softening of brain.
425	2/3	30	±	V.F.T.	>Tr.	1½.2.2½.2.2.1.½.0.0.0.	Neurosyphilis.
288	3/3	20	—	—	L.F.T. ?	1.1½.2.2.1½.1½.1½.1.1.0.	Sciatica.
271	3/3	20	±	—	Tr.	1.1.2.1.1.1.1.0.0.0.	Wasting of quadriceps.
457	3/3	30	V.F.T.	Tr.	>Tr.	1.1.1.1½.1½.1½.1.1.0.0.	*Petit mal.*
331	4/3	20	—	—	Tr.	½.1.1.2.2.2.2.1.1.0.	Amyotrophic lateral sclerosis.
389	4/3	25	F.T.	±	>Tr.	0.½.½.1.1½.1½.1½.½.0.0.	Organic cerebral disorder.

Although the results of the Lange reaction as expressed in figures and that of the M.T.R. as its 30 minutes value allow only of a fair comparison between the two tests, it will be noticed that the agreement is satisfactory in the majority of the 56 cases of this selected borderline material.

The tables show that we have not been able to solve all the difficulties and contradictions, especially as far as slight qualitative and quantitative changes in the protein composition of the C.S.F. are concerned, but we hope we have been able to demonstrate the value of the routine tests applied to the cerebrospinal fluid in " borderline " cases, and think that the collaboration between clinician and pathological laboratory might benefit from it. Such benefits might possibly be most valuable in clinically doubtful cases, and also in those cases where the clinical examination shows undoubted signs of organic disease, and the laboratory returns the results of the examination of the cerebrospinal fluid as " within the normal," while in reality it is slightly pathological.

Summary and Conclusion of Parts 1 and 2.

1. 122 cerebrospinal fluids, the overwhelming majority of them taken from mixed organic neuro-psychiatric material, have been investigated for cells, total protein, Nonne and Pandy, Lange and M.T.R. (W.R. and Meinicke have also been carried out).

2. The M.T.R. has been plotted as a " Time-Curve " and compared with the Lange.

3. The two tests were in agreement in 80–90 per cent. of the cases, varying in strength from negative to strong positive, with sedimentation in both reactions.

4. In 56 " organic " cases comparison of the two reactions with the other routine tests shows that quite a number of cerebrospinal fluids usually considered as " normal " are in fact pathological. The " normal " limits of the tests applied have been defined and discussed.

5. A careful comparative examination and evaluation of the different compounds of the C.S.F. should be of some help to the neurologist or psychiatrist in deciding the presence or absence of functional or organic conditions.

PART III.—THE LANGE AND MODIFIED TAKATA REACTION IN THE CEREBROSPINAL FLUIDS OF TYPICAL SCHIZOPHRENICS.

In the third and last part of this paper the experiences gained from the first two parts will be applied to the cerebrospinal fluids of about 40 typical schizophrenics.

When one gains more experience in psychiatry, one finds an increasing number of atypical cases among the so-called non-organic psychoses, and one gets more and more reluctant to make a diagnosis of schizophrenia unless the cases are very typical, or one has the opportunity of watching them for a long period. We have seen to an ever-increasing degree that schizophrenic syndromes are often superimposed on organic disturbances such as G.P.I., epilepsy, arteriosclerosis, high-grade mental deficiency, tumours of the brain and so on. Even if one can exclude organic conditions, there remains apparently a number of mixed psychoses which make classification difficult. It is true that heredity, and still more constitution, may act as a guide; but constitution is one factor only. Is it the decisive one? Let us take the example of tuberculosis, which not so long ago was considered hereditary or constitutional. There is no doubt about the importance of the asthenic type for this disease, but the person with a pyknic or athletic build may give way to tuberculosis, while an under-nourished asthenic may suffer from a simple bronchitis of non-tuberculous origin. I do not think it is proved beyond doubt that a cycloid personality may not acquire a schizophrenia and vice versa. Or take another example which will show the difficulty of clinical diagnosis; I refer to the change of symptoms which we have noticed during the last 10 or 20 years in G.P.I. The grandiose paralytic has become very rare. So have the classical cases of mania and perhaps of depression, and with regard to hysteria one may say that " la grande hystérie," except perhaps in wartime or similar " emergency " conditions, is usually not a hysteria but a commencing G.P.I. or disseminated sclerosis, a tumour of the frontal brain, or a schizophrenia, etc.

Since the therapeutic situation has improved, the necessity for making a proper diagnosis has become still more urgent and of greater practical importance. The

question of " in which condition should which treatment be applied," and what the prognosis will be, are in the minds of everyone who has to make a decision.

For the last two years investigations have been going on in this hospital with a view to seeing whether the laboratory can aid in establishing the diagnosis, especially that of schizophrenia.

The prospects and retrospects of such an approach are not very encouraging. About 30 years ago it was hoped that Abderhalden's dialysis method might give some help, but it came to nothing. About 20 years ago we had the era of permeability estimations, but when the method had been standardized, the differences between the individual non-organic psychoses became insignificant. Some years later, Zondek and his collaborators thought they could separate the manic-depressive group from the others by estimating what they thought was the bromide content of the blood. Unfortunately they only estimated hydrogen peroxide. Again, a few years later, Lehman-Facius claimed to have solved the problem by estimating special enzymes of a lipoid-splitting nature, but apparently the shaking of the reagent mixture was of greater importance than the kind of illness!

There has been, however, some quieter work going on in some laboratories on the proteins of the C.S.F. Kafka and his collaborators (1928) and Neel and his school (1933), working with different methods, found that in typical schizophrenics a disturbance of the C.S.F. protein exists, with the high probability of an increase in the so-called globulin fraction. The methods of these workers are somewhat complicated and have not become very popular. It is here that our investigations started. The methods we are using have been known for a very long time, and I want to make it clear from the beginning that our claims hold good only, at least for the time being, for typical active cases of schizophrenia, and also that the results are not of a specific but of a characteristic or typical nature. I have also to point out that the results can be used as a diagnostic help only in close collaboration with the clinician, who has to assure us that the patient is not suffering from an " organic " disorder. This relation between the clinician and the laboratory is, however, the usual one existing in all cases where the laboratory results are of a non-specific nature. An increase in the cell content, an increase in the proteins and a paretic Lange curve may be results given by quite different conditions. The mixing up of " typical " or " characteristic " with " specific " is one of the reasons why one of the reactions mentioned here, namely the Takata reaction, has been in disrepute for many years.

The method applied during the first phase of our investigations, apart from cell count, Nonne and Pandy reactions, was a Modified Takata Reaction. During the second phase, the Modified Takata Reaction has been transformed into a " time-curve " reaction and has been compared with the colloidal gold curve.

There are a few points which have to be emphasized. The Takata Reaction as applied to cerebrospinal fluid has not a very good reputation. This is due mainly to two factors:

1. To the disappointment that the reaction could not be used as a kind of Wassermann reaction as it was formerly claimed and hoped. This view arose from the mixing up of " characteristic " and " specific." A positive reaction is most likely due to an increase in the globulin fraction of the protein. This is one of the main characteristics of the changes found in the cerebrospinal fluid in syphilitic conditions, but it is not " specific " at all, and it will occur in other conditions affecting the central nervous system, and these are many.

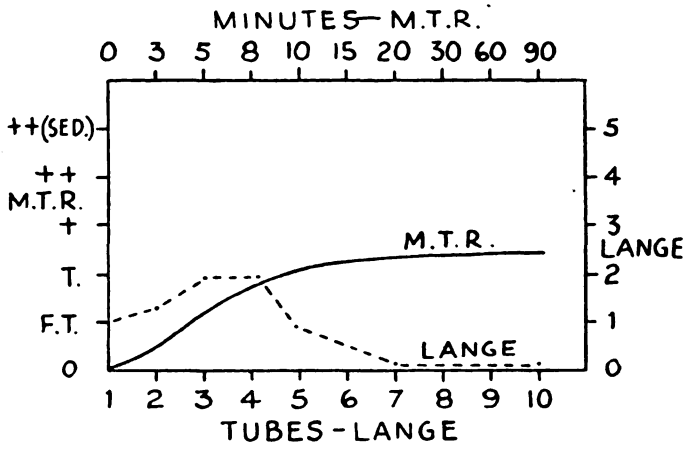
2. As the test is very easily performed, many people think that technical conditions can be ignored. The contrary is true, and with an old C.S.F. quite erroneous results may be obtained. This point has also been discussed by Lane.

In the first paper on the M.T.R. it had been stated that the reaction might be performed as a time-curve reaction. With the later work we made a closer observation of the development of the reaction, with time as one of the more important factors. So we started to put down the results of the M.T.R. as a time-curve reaction and to make the comparisons with the Lange curve. We began to overhaul our technique of reading and interpreting the gold-sol test, and made the rather unpleasant discovery that we had always misread and most likely misinterpreted the small changes in the test. If one wishes to read the Lange curve in a proper manner, one has to compare each tube with the control tube and also the test-tubes with each other. If this is done it will be found that what is usually read as

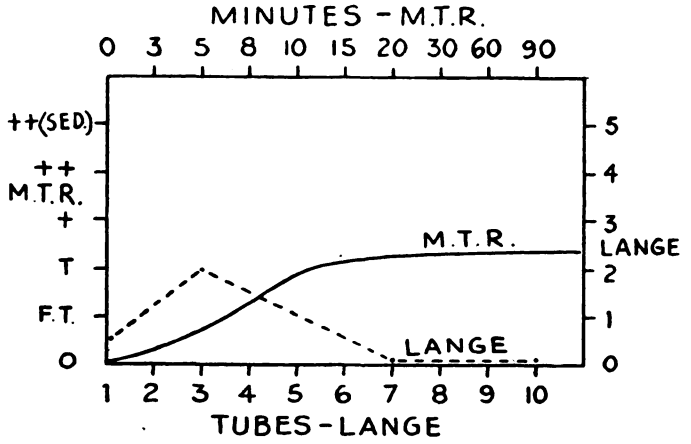
0.1.1.0.0.0.0.0.0.0. will often turn out to be ½.1.1½.1.½.0.0.0.0.0. The second unpleasant discovery was that a reading such as 0.1.2.1.0, which is usually considered as of no significance, is not normal at all, but slightly pathological. This is the outcome of about 250 examinations in which Lange, M.T.R., cell count, total protein and Nonne and Pandy reactions have been compared. Far more than half of the fluids came from patients suffering from organic disorders of the nervous system. I owe nearly all of the organic fluids to the kindness of Major Crawford and Major Hart-Mercer, R.A.M.C., and I wish to express my thanks to them.

I should like to show first some of the curves obtained on the organic and also on the negative non-schizophrenic control material. You will see that the curves of the M.T.R. and Lange show a certain agreement, although the Lange is a dilution curve obtained from 10 tubes, while the M.T.R. is a curve showing the development of a one tube reaction over a period of 90 minutes.* There is a very close agreement between the two curves in at least 80 per cent. of cases, and a reasonably good agreement in another 10 per cent.

I wish to show you now some curves obtained on schizophrenics:



CASE 397.—*Total protein:* 0·023 per cent. Nonne, ±; Pandy, ±. *Cells:* 3 lymphocytes in 3 c.mm.



CASE 430.—*Total protein:* 0·025 per cent. Nonne, F.T.; Pandy, ±. *Cells:* 5 lymphocytes, 1 endothelioid in 3 c.mm.

* A number of the curves published in the first part of this paper were demonstrated here.

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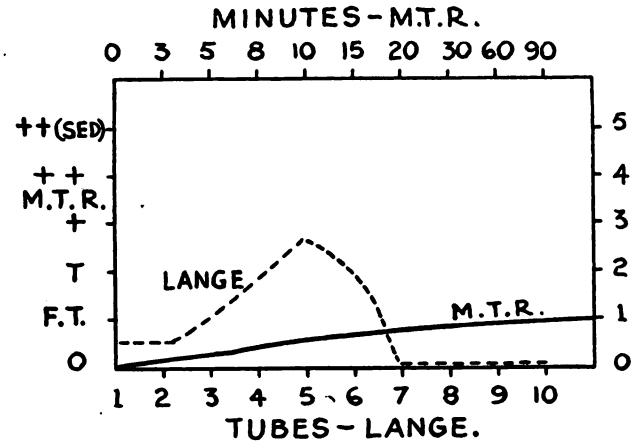
If you compare them with the negative or doubtful curves which you saw before you will agree that they are definitely different. They are of the GA type and of pathological significance. So far I have got curves from the fluids of about 40 typical schizophrenics, but it might be concluded by analogy that similar results would have been obtained in at least 40 to 45 out of the 53 fluids on which I had reported in a previous paper (1943).

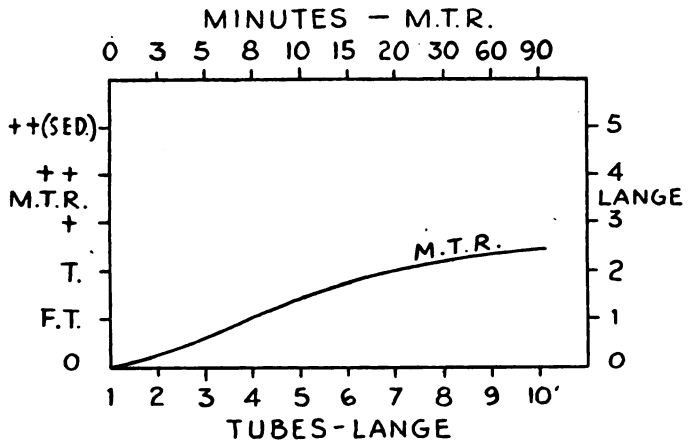
There is a possibility that feverish complications influence the M.T.R. to a higher degree than the Lange as seen in four cases.



CASE 297.—*Total protein:* 0·030 per cent. Nonne, T.; Pandy, ±. *Cells:* 2 lymphocytes, 1 polymorph in 3 c.mm. *Diagnosis:* Schizophrenia with P.U.O.

Although I would not like to commit myself to say more than that the results hold good only for cases of typical schizophrenia, I cannot resist the temptation to demonstrate a few cases where the laboratory has given a lead in establishing the diagnosis.

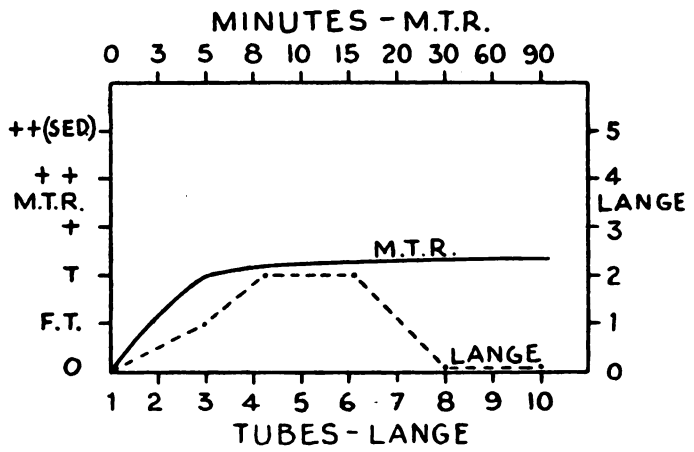
CASE 1.—This girl was admitted with a differential diagnosis of schizophrenia or a reaction of a schizoid, high-grade mental deficient personality on the birth of an illegitimate child. The patient was very impulsive on reception. She was transferred to the special treatment ward, and improved spontaneously to such an extent that the diagnosis of an active psychotic process was disregarded in spite of the results obtained from the examination of the C.S.F. (unfortunately only the M.T.R. was performed.)



CASE 362.—*Total protein:* 0·025 per cent. Nonne, negative; Pandy, negative. *Cells:* 3 lymphocytes, 3 polymorphs in 3 c.mm.

Only after another fortnight's observation on the ward and the occupational therapy department was it discovered that the girl was actively hallucinated, and later on other schizophrenic features became more pronounced.

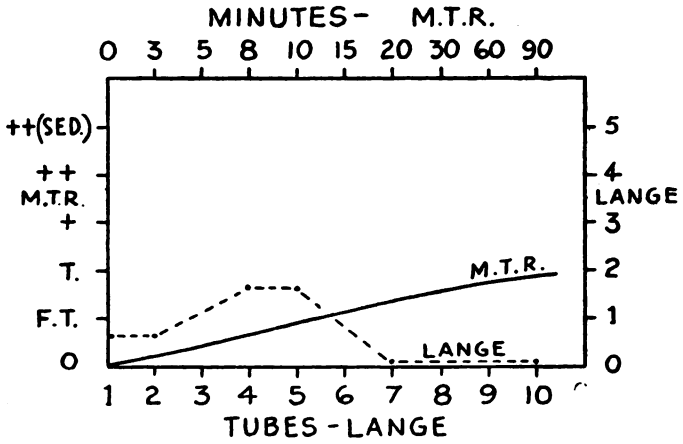
CASE 2.—The next case was similar. The results of the C.S.F. examination were as follows:



CASE 369.—*Total protein:* 0·030 per cent. Nonne, Trace; Pandy, ±. *Cells:* 2 lymphocytes in 3 c.mm.

On the special treatment ward the boy behaved in such a manner for a long time that the laboratory result was doubted. Later on, however, the boy became retiring, shy and actively hallucinated.

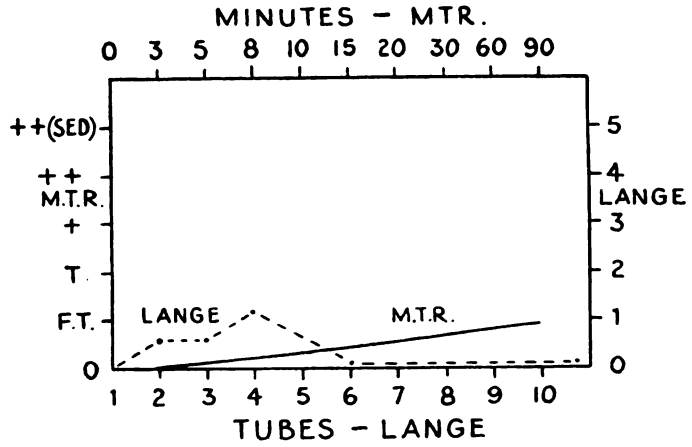
CASE 3.—This is a case of a girl who had been here under observation several months ago. We had come to the conclusion that she was suffering from a psychopathic personality, and a schizophrenia was excluded. She was discharged. She relapsed however, and was readmitted. Clinically there seemed to be no change. A lumbar puncture was performed in the hope of obtaining a normal C.S.F. The result in this respect was, however, disappointing, and you can see from the curve obtained that the fluid was slightly pathological.



CASE 375.—*Total protein:* 0·020 per cent. Nonne, negative; Pandy, negative. *Cells:* 5 lymphocytes, 4 polymorphs in 3 c.mm.

When examined again she admitted now that she had been suffering from ideas of reference and from auditory hallucinations for quite a while, but she had been so frightened by these experiences that she did not dare to talk about them when questioned previously.

CASE 4.—This girl was admitted with the diagnosis of hebephrenia. The results of the examination of the C.S.F. were as follows:



CASE 363.—*Total protein:* Insufficient for estimation. Nonne, negative; Pandy, negative. *Cells:* 3 lymphocytes in 3 c.mm.

A careful study of the history of the patient, and observation on the ward and the occupational therapy department, showed that the hebephrenic reaction on an unhappy love affair was superimposed on a high grade mental deficiency.

The previous findings that the M.T.R. is slightly positive in typical cases of schizophrenia has thus been confirmed on another series of such fluids, and the results are supported by slight pathological changes of the Lange reaction. As far as this test is concerned it had been claimed from time to time that it is slightly positive in the fluids of schizophrenics, since Galant-Ratner, from Bechterew's Institute, made the first contribution in 1924. The accuracy of such claims had been doubted by most workers in this field, and I myself stated in a previous paper (1943) that the gold-sol reaction is of little value in the diagnosis of schizophrenia. This statement, as you have just seen, has turned out to be wrong, after a more careful reading and evaluation of the test had been carried out.

The results obtained by the examination of the C.S.Fs. of typical schizophrenics by the use of the M.T.R. and Lange curve agree very well with those obtained by Kafka and Samson (1928), and by Neel and his collaborators (1933), using other methods. It means that the application of four different methods in examining the C.S.F. has proved that there are changes present which are not found in neurotics or typical manic depressives. I am a little reluctant to explain the nature of these changes, but after all we know they are indicative of a qualitative and/or quantitative disturbance in the so-called albumin-globulin ratio. Similar changes in the C.S.F. are very often found in degenerative processes of the central nervous system, and as you know, it has been claimed by various authors that slight disturbances of such a nature occur in the brains of schizophrenic patients. This has not been generally acknowledged. One objection is that cell atrophies of such kind are of a non-specific nature, and might have been caused by former illnesses or the disease preceding the death of the patient. I do not want to raise again the problem of specific and characteristic in this respect, and I am not a histopathologist, but I should like to mention that Kirschbaum and Heilbrun (1944) have just published a paper on pathological changes in the brains of schizophrenics where they think that the changes are not due to previous or intermittent disorders. So, even if the pathological deviations in these cases may not be of a so-called

"specific" nature, they have at least been obtained from brains of otherwise healthy schizophrenics. There is therefore a possibility that such findings on the brains and those obtained on the C.S.Fs. of schizophrenics have some connection with each other if they should be confirmed.

I wish to thank Dr. O. FitzGerald, Medical Superintendent of Shenley Hospital, for his encouragement to carry out these investigations and to publish the results.

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