FURTHER RESULTS WITH THE BOLTZ ACETIC ANHYDRIDE TEST.

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The recent recrudescence of interest in the Boltz test, as evidenced by Dr. C. J. Thomas's paper on the subject, prompts us to publish further results obtained with this test, together with a few conclusions as to its value.

Literature.

The test was first described by Boltz in 1923, and again in 1926. He found the test positive only in general paralysis and taboparesis. Grossman in 1925 confirmed Boltz's results, as did Harris in 1926. The latter believed it to be specific for general paralysis, and stated that malarial treatment had no effect upon it. In collaboration with Dr. J. P. Steel, one of us (J. E. N.) gave the earlier results obtained here, showing that the test did alter after malaria, wherein we were supported by Silverston in the same year. Other results were published by Fleming, and by Loberg. Walker and Sleeper, however, after investigation of the test, concluded that it was not specific at all.

In 1927 the test was again reported upon by Fleming, who found it invariably positive in paretic fluids. Schreus regarded it as a reliable test for syphilis, but Dietrich, Wullenweber, Scharfetter all obtained positive results in meningitis, the importance of meningeal involvement for a positive Boltz being also stressed by Cady. Baumann found it positive in 10% of psychopaths, and Duncan and Turnbull went so far as to state that it was positive in all samples of cerebro-spinal fluid, normal as well as abnormal. Greenfield and Carmichael, however, found it positive only in general paralysis and neuro-syphilis.

Further reports were published in 1928 by Nicole, Fleming,

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Novick, Myerson and Halloran—the last two concluding that the test was not of much value; and in 1929 by Ewing, Nicole, Pietrowski and Herbert. By this last the Boltz test is regarded as useless for the differentiation of general paralysis from other nervous diseases. Finally in 1930, Newman, and one of us (E. J. F.), upheld the value of the test, at least in mental hospital practice, while its use was further reported upon by Ewing, McCowan, Vincent and Thomas.

As far as it is possible to summarize the views of so many observers, it might be said that in the earlier stages considerable reliance was placed upon the test, and it was regarded as specific in varying degrees in that it was thought to be a reliable indicator of either (1) general paralysis, or (2) syphilis of the nervous system in general, or at least (3) conditions of meningeal involvement. More recently considerable doubt has been cast upon its value. It has been described as running parallel with the globulin tests in its results, and has been said to be dependent upon a protein increase.

Technique and Reagents.

The technique has often been described, and little need be said about it here. As regards reagents, we have used only two samples of acetic anhydride, both of which have yielded good results. We would emphasize this fact in view of the possible chemical basis of the test, which will be discussed later. The only uncertainty we have encountered has been in connection with the ageing of the reagent. After using the same sample of acetic anhydride for eighteen months or two years the colour reaction tends to become weaker. The dirty brown colour so often referred to by other writers we have considered as negative, though we are not entirely convinced of the wisdom of invariably doing this.

Material.

The material on which our conclusions are based consists of 890 fluids, including 356 from cases of general paralysis. Of the paretic fluids, 176 were examined before and 180 after malarial treatment. The time elapsing between the malaria and the serological examination has varied from one month to 7½ years, reckoning such time period (in those cases that had more than one course of malaria) from the date of the first course.

The Boltz test was accompanied in every case by a cell count,

a globulin reaction, and a Lange's colloidal gold test. In 111 instances a protein estimation was made, and sometimes certain other chemical tests, to be described later.

General Results.

(1) *Non-paretic Fluids.*

In the 534 non-paretic fluids, the Boltz was negative in 98·67%, positive results occurring in only 7 fluids. Of these, 3 were definitely luetic in type, and 2 others were from cases showing symptoms suggestive of meningeal involvement. It is the latter type of case that is probably responsible for many writers (*e.g.*, Herbert) belittling the test as a means of differentiating general paralysis from other conditions, but in mental hospital practice these non-paretic positives are so rare that we think the test is still of value.

Table I.

General paralysis before treatment. Lange paretic in all cases.								
Globulin +, Boltz +.	Globulin +, Boltz −.	Globulin −, Boltz +.	Globulin −, Boltz −.	Total globulin +.	Total globulin −.	Total Boltz +.	Total Boltz −.	Total fluids.
141	32	2	1	173	3	143	33	176

(2) *Untreated General Paralysis.*

The results of testing 176 fluids from untreated cases of general paralysis showed conclusively that though a positive Boltz may be a strong indicator of general paralysis, a negative Boltz is of no significance, for no less than 33 fluids (18·75%) were negative. Interestingly enough, we have come across several cases where a negative Boltz was obtained before malaria, whereas *very soon* after malaria a positive result occurred.

(3) *General Paralysis after Malaria.*

Here the results were very interesting. In these 180 cases the proportion of negative results to the total fluids was decidedly

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high, namely, 61·11%, and the percentage was higher in examinations carried out several years after malaria. Thus in 123 fluids tested within three years of malaria, 48·78% were Boltz-negative, whereas in 57 fluids examined over three years after malaria 89·12% were negative.

TABLE II.

	Not general paralysis.									General paralysis after treatment.								
	Globulin +, Boltz +.	Globulin +, Boltz −.	Globulin −, Boltz +.	Globulin −, Boltz −.	Total globulin +.	Total globulin −.	Total Boltz +.	Total Boltz −.	Total fluids.	Globulin +, Boltz +.	Globulin +, Boltz −.	Globulin −, Boltz +.	Globulin −, Boltz −.	Total globulin +.	Total globulin −.	Total Boltz +.	Total Boltz −.	Total fluids.
Highest figure of Lange not above— 1	..	15	..	338	15	338	..	353	353	..	9	1	23	9	24	1	32	33
2	2	30	..	88	32	88	2	118	120	..	10	..	11	10	11	..	21	21
3	3	20	1	21	23	22	4	41	45	2	11	2	10	13	12	4	21	25
4	1	9	..	5	10	5	1	14	15	10	12	2	4	22	6	12	16	28
5	..	1	..	..	1	..	..	1	1	41	19	12	1	60	13	53	20	73
Total . .	6	75	1	452	81	453	7	527	534	53	61	17	49	114	66	70	110	180

As to the question of repeated attacks of malaria, the time periods have, as we said before, been calculated from the date of the first course and although there was a trifling preponderance of negative results in cases repeatedly treated as compared with those who had one course only, the difference was too small to be considered significant. The same remark applies to the use of adjuvants to malarial treatment, such as neosalvarsan, stovarsol, bivatol and bismuth preparations.

COMPARISON WITH OTHER TESTS.

(*a*) *Globulin.**—The globulin was positive in 15·17% of non-paretic fluids, 98·30% of paretic fluids before treatment, and 63·33% of fluids from treated cases. It at once becomes apparent that the two tests by no means give parallel results. The discrepancy is most marked in the case of the treated patients; these we propose to discuss in greater detail.

The disagreement is of two kinds—positive globulin with negative

* The test used was Pandy's.

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Boltz and negative globulin with positive Boltz. Comparing these two, we know that the ratio of total positive globulins to negative globulins is roughly 2 to 1. Hence, if the disagreement were evenly distributed between the two groups, we should expect the number giving positive globulin with negative Boltz to be approximately twice the number giving negative globulin with positive Boltz. This is provided that the total positive Boltz were to the total negatives as 1 to 2. Actually, they were slightly more; hence the number of positive globulins with a negative Boltz should be less than twice the number of negative globulins with a positive Boltz.

TABLE III.

General paralysis after treatment.

Years since malaria.	Globulin +, Boltz +.	Globulin +, Boltz −.	Globulin −, Boltz +.	Globulin −, Boltz −.	Total globulin +.	Total globulin −.	Total Boltz +.	Total Boltz −.	Total fluids.	Highest figure of Lange not above— 1	2	3	4	5	Total.
0—1	32	20	9	5	52	14	41	25	66	3	3	6	11	43	66
1—2	10	9	7	8	19	15	17	17	34	5	2	6	2	19	34
2—3	6	9	..	8	15	8	6	17	23	2	5	3	8	5	23
3—4	4	10	..	7	14	7	4	17	21	2	2	7	5	5	21
4—5	1	4	1	8	5	9	2	12	14	7	5	1	1	..	14
5+	..	9	..	13	9	13	..	22	22	14	4	2	1	1	22
Total .	53	61	17	49	114	66	70	110	180	33	21	25	28	73	180

Actually, however, the proportion was just over 3 to 1. This shows that the disagreement was not evenly and proportionately distributed between the two groups, but that it was 50% higher—a significant figure—for the positive globulins with a negative Boltz than for the negative globulins with a positive Boltz. This may be of interest in view of the chemistry of the test.

(*b*) *Lange's colloidal gold.*—For purposes of classification we have grouped our Lange results according to the highest figure found in the curves. In the non-paretic series there were two positive Boltz with a Lange going to a 2, four with a Lange of 3 and one with a Lange of 4. In the untreated paretics all the Lange curves were typically paretic.

After treatment we again find much disagreement, more especially

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with the higher Lange readings. Thus with the Lange going no higher than a 3 we obtained 6·33% *positive* Boltz (79 cases), whereas in the 101 cases with a Lange of 4 and 5 there were 36% *negative* Boltz. We should remember, however, that we have many more negative Boltz over three years after malaria than under, and as, of the 57 Lange done over three years after malaria, only 13 showed a reading of more than 3, very little further calculation will show that the disagreement between the two tests is more significant than might at first sight appear. Nevertheless, this disagreement is less marked here than it is between the Boltz and the globulin tests; and, for that matter, than between the globulin and the Lange. For instance, the percentage of positive globulins with a Lange of 3 and under was 40·5, compared with 82% with a Lange of 4 and 5. The corresponding figures for the Boltz test were 6·33% and 64% respectively.

(*c*) *Syphilitic tests.*—Inasmuch as the Boltz test is a simple chemical one, not much relation is to be expected between it and specific tests for syphilis, nor have we found any, except such as is merely due to the fact that the specific tests are indicative of a disease that *may* lead to such chemical changes as would give rise to a positive Boltz. We have numerous cases where the two disagree entirely, the Boltz being frequently negative in presence of positive specific tests. Four cases may be given as typical examples.

1. *Cerebro-spinal fluid.*—Cells *nil*, globulin positive, Boltz negative, protein 65, Lange 000122100, Wassermann positive, Sachs-Georgi positive, Sachs-Witebsky positive, Meinicke positive.
Blood.—Wassermann doubtful, Sachs-Georgi positive, Sachs-Witebsky positive, Meinicke positive.

2. *Cerebro-spinal fluid.*—Cells *nil*, globulin positive, Boltz negative, protein 35, Lange 1234543110, Wassermann positive, Sachs-Georgi positive, Sachs-Witebsky positive, Meinicke positive.
Blood.—Wassermann 1 in 45, Sachs-Georgi positive, Sachs-Witebsky positive, Meinicke positive.

3. *Cerebro-spinal fluid.*—Cells *nil*, globulin negative, Boltz negative, protein 20, Lange 0011211000, Wassermann positive, Sachs-Georgi doubtful, Sachs-Witebsky doubtful, Meinicke negative.
Blood.—Wassermann doubtful, Sachs-Georgi negative, Sachs-Witebsky negative, Meinicke negative.

4. *Cerebro-spinal fluid.*—Cells 30, globulin positive, Boltz negative, protein 25, Lange 4555554321, Wassermann positive, Sachs-Georgi doubtful, Sachs-Witebsky doubtful, Meinicke positive.
Blood.—Wassermann 1 in 15, Sachs-Georgi doubtful, Sachs-Witebski positive, Meinicke positive.

Chemistry of the Test.

Boltz's original suggestion was that the test depended upon the cholesterol content of the cerebro-spinal fluid, and in this he was to some extent supported by Grossman, Harris, Greenfield and Carmichael, and Cady. Weston, who in 1915 and 1917 showed the cholesterol content to be raised in meningeal disease, proved that the Boltz test may be negative even in presence of an increase of cholesterol.

In 1926 Walker and Sleeper stated that the test was not specific at all, and that it was due to a reaction between the proteins of the fluid and an aldehyde present as an impurity in the acetic anhydride used. In 1927 Blix and Backlin also pointed out that the test was a protein test, not unlike the original Adamkiewicz reaction, and that it was due to tryptophane. Duncan, Fleming and Herbert have all supported the view that the test is a glyoxylic acid one, and that it must be proportional to the total protein content of the cerebro-spinal fluid.

We have been engaged recently in testing the reaction of numerous samples of cerebro-spinal fluid to various colour tests for proteins, more especially those indicative of the carboxyl and α-amino groups, the phenyl group, the hydroxy-phenyl (tyrosine) group and the indolyl (tryptophane) group. The tyrosine tests yielded no results of interest, but with the tryptophane tests we were amply able to confirm the fact that the Boltz test and the tryptophane radicle went hand in hand. Especially was this evident from the use of the Hopkins-Cole reaction. This particular test was employed on 69 fluids, and in every case where the Boltz was positive the Hopkins-Cole reaction was strong, while where the Boltz was negative the Hopkins-Cole gave only a very weak coloration.* But on the other hand, in one or two instances we got a strong Hopkins-Cole reaction with a negative Boltz. These negative Boltz were all of that dirty brown colour which most authors (*e.g.*, Levinson in his book) agree to call negative. But we strongly suspect that it may not be wise always to do this without further investigation, and that though most of these brown colours are definitely negative reactions, yet a few may be positive. This brown colour is probably due to

* The reagent was prepared by the action of 100 c.c. of a saturated solution of oxalic acid on 6 grm. of a 10% sodium amalgam, filtering after the production of gas had ceased. In every case the reagent was used undiluted, and also diluted with one, two, and three volumes of water. The actual test was performed as for the Boltz, namely, 1 c.c. of fluid, 0·3 c.c. of the reagent, mixing, and then adding 0·8 c.c. of pure sulphuric acid drop by drop.

the heat developed by the addition of the strong sulphuric acid to the acetic anhydride. The heat is necessary for the positive lilac colour to develop, but it may be so excessive as to cause a charring of organic matter which might conceal the true positive reaction, especially if it be a very weak one. This disadvantage may sometimes be obviated by a modification of technique, thus: Add the sulphuric acid to the fluid very slowly, keeping the mixture cool, then cool down to room temperature, add the acetic anhydride, and then heat slowly on a water-bath. By this means it is possible to limit the development of the brown colour due to charring, and a weakly positive reaction may be recognized, and even, by this process of slow heating, intensified.

In this connection we would emphasize the fact that a faint lilac coloration may be missed when looking *through* the tube against a white background, though it may still be recognizable on looking *down into* the tube held *over* a white background.

The charring referred to above does not, however, occur when using the Hopkins-Cole reagent. This fact alone would urge us to recommend that if such a test as the Boltz be retained as a routine measure, then it should be used in the form of a Hopkins-Cole reaction. This would also have the advantage that one would be using a known reagent of standard strength rather than relying upon a mere impurity present in uncertain amounts in the acetic anhydride used for the Boltz test.

It has been said that, being a protein test, the Boltz must, therefore, go parallel in intensity with the protein content of the cerebro-spinal fluid. If that were so, then, as the Hopkins-Cole reaction is chemically the same as the Boltz, by standardizing the former reagent it should be possible to obtain such varying intensities of colour reactions that these might be made the basis for a roughly quantitative test. This we have tried on some 50 fluids, and in 48 of these we were able to prognosticate the amount of total protein present within 10 mgrm. per 100 c.c. Our two exceptions, however, were so glaring, that we came to cast very definite doubt upon this alleged relationship between protein content and the Boltz test.

Although we have not a very large series of protein estimations compared with the Boltz test, we append our results in the form of a table, from which it will readily be seen that, as one of us (E. J. F.) has already suggested, unless it be a matter of protein proportions in the fluid, total protein content would certainly not explain the Boltz test.

TABLE IV.

Protein in mgrm. per 100 c.c.	Not general paralysis.					General paralysis before treatment.					General paralysis after treatment.					All cases.				
	Globulin +	Globulin −	Boltz +	Boltz −	Total.	Globulin +	Globulin −	Boltz +	Boltz −	Total.	Globulin +	Globulin −	Boltz +	Boltz −	Total.	Globulin +	Globulin −	Boltz +	Boltz −	Total.
0–20	3	..	..	3	3	..	..	..	..	..	3	9	..	12	12	6	9	..	15	15
20–30	7	2	..	9	9	3	..	1	2	3	10	8	3	15	18	20	10	4	26	30
30–40	4	..	..	4	4	2	..	1	1	2	9	2	1	10	11	15	2	2	15	17
40–50	3	..	1	2	3	9	..	4	5	9	9	..	1	8	9	21	..	6	15	21
50–60	..	..	..	..	..	8	..	6	2	8	4	..	2	2	4	12	..	8	4	12
60–70	..	..	..	..	..	2	..	..	2	2	1	..	..	1	1	3	..	..	3	3
70–80	..	..	..	..	..	4	..	4	..	4	..	..	..	..	..	4	..	4	..	4
80–90	..	..	..	..	..	3	..	3	..	3	..	..	..	..	..	3	..	3	..	3
90–100	..	..	..	..	..	1	..	1	..	1	1	..	1	..	1	2	..	2	..	2
Over 100	2	..	1	1	2	2	..	1	1	2	..	..	..	..	..	4	..	2	2	4
Total	19	2	2	19	21	34	..	21	13	34	37	19	8	48	56	90	21	31	80	111

Should we then look for an increase of only *certain* proteins in the cerebro-spinal fluid—presumably those proteins that are particularly rich in the indolyl group? And if so, what are those proteins?

Now it has recently been stated by Ohlssen that globulins have a specially high tryptophane content. But this, if true, would lead us to expect that the Boltz test would be positive whenever the globulin was increased, and we have already seen how this is far from being the case. It might, of course, be argued that even though the globulin were moderately increased—enough to give a positive globulin test—yet it might not be sufficient to give a positive Boltz unless the other proteins were increased too—in other words unless both the globulins and total proteins were increased. Here, again, we come across instances that contradict these suggestions. Let two typical instances suffice:

1. Globulin negative, Boltz positive, Hopkins-Cole very strong, total protein 30 mgrm. per 100 c.c.
2. Globulin positive, Boltz negative, Hopkins-Cole very weak, total protein 50 mgrm. per 100 c.c.

Might it then be shown whether the real factor on which the Boltz test depends is the *relative* amounts of *different* proteins in the cerebro-spinal fluid—in other words the protein partition? It is already largely believed that the Lange depends upon this factor. But this would mean that whenever the usual proportion of 7 of

albumen to 1 of globulin altered to the paretic kind of ratio, namely about 2 of albumen to 1 of globulin, then both the Lange and the Boltz should give a positive reading. We have, it is true, already noted the fact that the Boltz test on the whole—especially after malaria—agrees more (or should we say disagrees less) with the Lange than it apparently does with any of the other tests, but even so the agreement is only very partial, and many exceptions occur that are difficult, if not impossible, to explain satisfactorily.

Perhaps, after all, we may have to return, in part at least, to the cholesterol content for an explanation. Cholesterol binds up very tightly with globulin, and it is only with difficulty removed from cholesterol-globulin mixtures. It is therefore possible that both protein and cholesterol contents are of importance. But here, of course, is where we must leave the matter. Our results disprove more than they prove anything, but inconclusive as they may be, we venture to think that they do show that it is not simply a matter of total proteins, or globulin increase, or protein partition, or cholesterol alone, but probably a combination of factors the elucidation of which will have to be carried out by investigators more skilled and learned than we are.

If this complex of factors be identified, it will be interesting to see whether it exists in certain diseases (say general paralysis) and not in others, and if this were shown to be so, the Boltz test—or some modification of it—might yet be rehabilitated to a position of some diagnostic importance in the serological investigation of nervous disorders.

Conclusions.

(1) The Boltz test is rarely positive in non-paretic cases except in certain conditions of meningeal involvement, such as are but infrequently found in mental hospital work.

(2) It is not invariably positive in general paralysis, and a negative Boltz is of little diagnostic value.

(3) It agrees with no other usual test, though it probably disagrees less with the Lange than with, say, the globulin reactions.

(4) It readily becomes negative after malarial treatment, but mostly so after some years have elapsed since the first attack of malaria.

(5) The test does not depend only on the globulin content of the cerebro-spinal fluid, or on the total protein increase, or the protein partition, though it probably does bear a relationship to the total

tryptophane value of the fluid proteins, and perhaps also to cholesterol.

(6) If the test is to be further investigated—and we think it should—it would be infinitely preferable to use it in its Hopkins-Cole form, because (*a*) the Hopkins-Cole reagent can be standardized, (*b*) the colour reactions can be graded so as to render it quantitative, and (*c*) there is not the brown charring found when using acetic anhydride.

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