THE ACETIC ANHYDRIDE TEST IN CEREBROSPINAL FLUID.

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THE object of this paper is to place on record the results of the sulphuric acetic anhydride test in a number of cerebrospinal fluids, and further to discuss the possible underlying 'causal factor.'

The work has been done in the Pathological Laboratory of the National Hospital, Queen Square, and we desire here to tender our thanks to the physicians of the hospital for permission to publish these reports. In all, the series includes 50 cases ranging from non-organic conditions of the central nervous system to dementia paralytica.

LITERATURE.

In 1923 Boltz¹ published a paper on "Studies of the Cerebrospinal Fluid with an Acetic-anhydride Sulphuric Acid Test." This is, so far as we have been able to ascertain, the first paper upon this subject. In examining a large series of cases he found that the reaction gave a positive finding only in cases of dementia paralytica and taboparesis. He had the opportunity of examining 22 fluids from cases of dementia paralytica, and in every one found a positive result; in two cases of taboparesis, one gave a positive, the other a slight positive. No other positive reactions were obtained. Discussing the 'causal factor,' he states that the substance is not destroyed by heating the cerebrospinal fluid, and assumes therefore that it cannot be analogous to the substance giving the colloidal gold reaction. Further, he found that the 'causal factor' was thrown down with the globulin in Noguchi's test, as after so doing the supernatant fluid gave a negative result, while a saline solution of the centrifuged deposit gave a positive result. He lastly argued that though the test is similar to a cholesterol reaction its positivity in such cases "cannot be due to excess of cholesterol, as Weston states that the cholesterol content in the cerebrospinal fluid of dementia paralytica is not as high as that in cases of other forms of cerebral degeneration."

Grossman², in 1925, found the test to be positive in 28 cases of general paralysis in a series of tests upon 60 cases. The remainder of the cases, all of which gave negative results, were those of patients suffering from psychoses, epilepsy and other non-syphilitic conditions. In opposition to Boltz he states that the reaction is dependent upon the cholesterol content of the spinal fluid.

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It is closely similar to the reactions of Salkowski and Liebermann-Burchard, by which the cholesterol content of various body fluids is determined.

Harris³ in the early part of the present year recorded a further series of cases to which this test had been applied. He, too, found that it was specific for general paralytic fluids. In 92 cases of general paralysis he found this test positive in 84 (91 per cent.) ; five cases of neurosyphilis were examined, two of which gave a positive result, and from 83 cases of other types of mental disorder he obtained only one positive. He made a further interesting observation that malarial treatment, though changing the strength of the Wassermann reaction, did not affect the acetic anhydride test. From his observations he concluded that in general paralysis this test was almost as reliable as the Wassermann reaction, and he suggested the possibility of the reaction depending upon the presence of cholesterol in the cerebrospinal fluid.

Steel and Nicole⁴ have published their results of this reaction upon fluids of a large series of general paralytics and non-syphilitic patients. They have only recorded the results of three reactions, the Lange, the globulin, and the acetic anhydride. In 39 fluids from non-syphilitic patients no positive reaction with acetic anhydride was obtained. In 36 fluids from parietic patients they only obtained one negative result ; the globulin reaction was also negative in this isolated case. In eight fluids out of a total of 30 from patients suffering from general paralysis who had had malarial treatment the result of the acetic anhydride test was negative. They state that this reaction is more sensitive than the globulin reaction, and remains unaltered by malarial treatment for a longer period than the globulin or colloidal gold reaction.

TECHNIQUE.

We have used the following technique in performing this test. The cerebrospinal fluid used was never over 48 hours old and had not been heated ; if the test could not be performed at once the fluids were kept in an ice chest. Our experience has shown us that fluids which give a positive result may be kept for a week or more in the ice-chest without any change in the result of the test when repeated.

One c.c. of the cerebrospinal fluid is measured into a test-tube with a graduated pipette. To this is added 0.3 c.c. of *chemically pure* acetic anhydride. The test-tube is then gently agitated to cause an emulsion to form. To this is then added drop by drop 0.8 c.c. of chemically pure sulphuric acid. Should no colour immediately appear, the test-tube is again shaken gently when invariably some colouration takes place.

Our experience has shown us that in almost every instance a change in colour begins to appear after 0.6 c.c. of sulphuric acid has been added and that the consequent addition of a further 0.2 c.c. has merely intensified the original colour. Further, we have observed that unless a reading be immediately taken after the addition of the sulphuric acid further colour changes may take

place and give a wrong result. In other words, the reaction is a rapid and fleeting one, and requires immediate recording.

A positive result is constituted by a very definite lilac colour—it may range from a comparatively light to a deep lilac. Other colours such as pink, blue-pink or brown were considered negative. In fact, any colour other than a definite lilac must be considered without dubiety as negative. Our experience has led us to associate a dirty brown colour with a high protein content.

In our series we have also made a routine examination of all the fluids. Our records thus not only include the results of the acetic anhydride test but also the physical characters of the fluid, the cell content, the total protein and the Wassermann reaction in the spinal fluid of all and in the blood of most of the cases. We are thus in a position to discuss whether a positive acetic anhydride test is constantly associated with a positive result from one of the other reactions or whether it may be found only in cases which show a positive result in all reactions: or whether it may be due to some 'causal factor' so far not found to influence the usual routine reactions, the causation of which is known.

RESULTS.

Our results are given in the table. The diagnosis of each case has been made upon clinical grounds and is not the result of biochemical and biological criteria.

G.P.I. FLUIDS

	<i>Ser.</i>	<i>Acetic Anhy- dride Test.</i>	<i>Physical Charac- ters.</i>	<i>Cells.</i>	<i>Total Protein.</i>	<i>Nonne- Apelt.</i>	<i>Pandy</i>	<i>Lange.</i>	<i>C.S.F. W.R.</i>	<i>Blood W.R.</i>
E.B.	M.	Deep lilac.	Clear, colour- less.	32· 10% large mono.	0·1%	pos. +++	pos. +++	2344533221	4444	4443
G.S.	M.	Deep lilac.	Clear, colour- less.	15% poly. 45·0 25% large mono.	0·075%	pos. +++	pos. +++	3533432100	4444	44??
A.P.	M.	Lilac	Slightly yellow.	40·0 10% large mono.	0·1%	pos. +++	pos. +++	5555555543	4444	4210
W.	M.	Lilac	Clear, colour- less.	14·0	0·06%	pos.	neg.	3321000000	4444	4444
R.	M.	Deep lilac.	Clear, colour- less.	40·0 5% large mono.	0·045%	pos. +	pos. +	4555554443	4444	4444
G.B.	M.	Brown lilac.	Clear, colour- less.	15·0 15% large mono.	0·08%	pos. +++	pos. +++	5555555432	4440	neg.

	<i>Sex.</i>	<i>Acetic Anhydride Test.</i>	<i>Physical Characters.</i>	<i>Cells.</i>	<i>Total Protein.</i>	<i>Nonne-Apelt.</i>	<i>Pandy</i>	<i>Lange.</i>	<i>C.S.F. W.R.</i>	<i>Blrod W.R.</i>
L.I.	M.	Lilac	Clear, colourless.	6·0	0·05%	pos. +++	pos. +++	3200455555	4444	4443
W.R.	M.	Lilac	Clear, colourless.	82·0 5% large mono.	0·1%	pos. +++	pos. +++	3445555432	4444	—
H.P.	M.	Lilac	Clear, colourless.	7·0	0·065%	pos. ++	pos. ++	4555555533	4442	4443
A.L.	M	Lilac	Clear, colourless.	60·0 10% large mono.	0·10%	pos. ++	pos. ++	3555444443	4444	4443
R.F.	M.	Lilac	Clear, colourless.	36·0	0·09%	pos.	pos.	5555555553	4444	3200
S.	M.	Lilac	Clear, colourless.	8·5 5% large mono.	0·06%	pos. ++	pos. ++	2444555543	4443	4444
B.	F.	Lilac	Clear, colourless.	14·0 4% large mono.	0·045%	weak pos.	weak pos.	0012100000	1000	—
S.	M.	Lilac	Yellow	57·0 5% large mono.	0·09%	pos. ++	pos. +++	4555554332	4442	—
F.	M.	Lilac	Clear, colourless.	45·0 5% large mono.	0·06%	pos. ++	pos. ++	5555554211	4444	4443
M.	F.	Lilac	Clear, colourless.	75·0 15% large mono.	0·075%	pos.	pos. ++	3444333221	4444	4443
FLUIDS OF TABES DO RSAL IS.										
H.	M.	Pink-blue.	Clear, colourless.	1·0	0·45%	very weak pos.	pos. +	No change	neg.	neg.
J.	F.	Yellow	Clear, colourless.	19·0	0·03%	weak pos.	pos.	2233443322	neg.	pos.
M.	M.	Pink	Clear, colourless.	45·0 10% large mono.	0·1%	pos. ++	pos. ++	0232221110	4444	4444
S.	M.	Lilac pink.	Clear, colourless.	1·0	0·04%	neg.	neg.	0001110000	neg.	neg.

	<i>Sex.</i>	<i>Acetic Anhydride Test.</i>	<i>Physical Characters.</i>	<i>Cells.</i>	<i>Total Protein.</i>	<i>Nonne-Apelt.</i>	<i>Pandy</i>	<i>Lange.</i>	<i>C.S.F. W.R.</i>	<i>Blood W.R.</i>
G.	F.	Light pink.	Clear, colourless.	3·0	0·03%	neg.	neg.	0001101000	neg.	neg.
S.	M.	Yellow	Clear, colourless.	52·0 8% large mono.	0·04%	neg.	neg.	0011000000	4200	4442
FLUIDS OF OTHER FORMS OF SYPHILIS.										
S.J.	M.	Lilac	Yellowish turbid.	85·0 10% poly, 5% large mono	0·8%	pos. +++	pos. +++	0000011112	4442	4433
F.	M.	Bright pink.	Clear, colourless.	36·0	0·09%	pos.	pos.	5555555553	4444	3200
R.	F.	Light lilac-pink.	Clear, colourless.	56·0 5% large mono.	0·045%	neg.	weak pos	0000100000	4421	?
B.	M.	Light lilac-pink.	Clear, colourless.	20·0	0·03%	neg.	very weak pos.	4444332100	4443	4433
OTHER CONDITIONS.										
TUMORS OF BRAIN AND COORD.	URS F.	Brown	Clear, colourless.	?	0·02%	neg.	neg.	—	neg.	neg.
J.	M.	Pink	Slight yellow.	?	0·03%	neg.	neg.	1122200000	neg.	neg.
L.	F.	Pink	Clear, colourless.	1·0	0·15%	pos. +	pos. +	0001122110	neg.	neg.
Q.	M	Brown	Clear, colourless.	0	0·03%	weak pos.	weak pos.	0002333000	neg.	neg.
L.	M.	Pink	Clear, colourless.	0	0·065%	weak pos.	weak pos.	—	?	?
S.	F.	Brown	Slightly yellow.	2·0	0·09%	pos. +	pos. +	No change	neg.	neg.
C.	M.	Pink	Clear, colourless.	3·0	0·1%	pos.	pos.	0000012221	neg.	neg
M.	M.	Brown	Yellow & coagul'm	5·5	0·8%	pos.	pos.	0000111233	neg.	neg.

	Sex.	Acetic Anhydride Test.	Physical Characters.	Cells.	Total Protein.	Nonne Apelt..	Pandy	Lange.	C.S.F. W.R.	Blood W.R.
SUBA B.	CUTE F.	E COMBI Pink	NED DEGENERATION. Clear, colourless.	0	0.03%	neg.	very weak pos.	No change	neg.	neg.
T.	F.	Brown	Clear, colourless	0	0.025%	neg.	neg.	3444200000	neg.	neg.
LEPRA D.	NEURITIS. F.	Brown	Clear, colourless.	3.0	0.06%	weak pos.	pos.	No change	neg.	neg.
ENCF W.	PHALITIS LE M.	Pink	THARGIC A (CHRONIC). Clear, colourless.	9.0	0.04%	neg.	neg.	—	neg.	neg.
VASC K.	ULAR. M.	Brown	Clear, colourless.	0	0.025%	neg.	neg.	No change	neg.	neg.
DISSE S.	MINA F.	TED SCI Yellow	EROSIS. Clear, colourless.	3.0	0.02%	neg.	weak pos.	No change	neg.	neg.
K.	F.	Pink	Clear, colourless	4.0.	0.03%	weak pos.	weak pos.	0112110000	1000	1000
W.	F.	Pink	Clear, colourless.	20.0 10% mono.	0.035	pos. +	pos. +	5454331100	neg.	neg.
MENI L.	NGITIS M.	Pink	Yellow and coagul'm	++ poly	1.0%	pos. +++	pos. +++	—	—	—
CERE C.	BRO-F.	MACULAR Light lilac	DEGENERATION. Clear, colourless.	1.0	0.02%	neg.	neg.	No change	neg.	neg.

FLUID OF NON-ORGANIC CASES.

G.	M.	Light pink lilac.	Clear, colourless.	4.0 1 endothelial.	0.04%	neg.	weak pos.	No change	neg.	neg.
B.	F.	Light pink.	Clear, colourless.	3.0	0.025%	neg.	neg.	No change	neg.	neg.
M.	M.	Light pink.	Clear, colourless.	1.0	0.02%	neg.	neg.	—	neg.	neg.
B.	F.	Brown pink.	Clear, colourless.	0	0.025%	neg.	neg.	—	neg.	neg.
S.	M.	Brown pink.	Clear, colourless.	4.0	0.025%	neg.	neg.	No change	neg.	neg.
H.	M.	Brown pink.	Clear, colourless.	0	0.025%	neg.	neg.	—	neg.	—

Of 16 cases of dementia paralytica the acetic anhydride test was positive in 15, the remaining fluid gave a dirty brown-blue colour, which was considered negative. On examination of the table it will be seen that except for one of these cases the fluids were all clear and colourless; that the cell counts varied from 82 to 6 per cmm., one fluid containing a few polymorphs; that the total protein varied from 0.1 to 0.045 per cent.; that the Nonne-Apelt reaction was positive (+ to ++++) in all; that the Pandy reaction was positive in all except one; that the Lange gold-sol curves were all of the paretic type except two; that the Wassermann reaction was positive in all the fluids and in all but one of the bloods examined. It is therefore manifest that all the cases examined gave a typical cerebrospinal fluid picture of dementia paralytica.

Cases of tabes dorsalis gave various colour reactions varying from yellow to lilac pink; but no fluid exhibited an undoubted general paralysis reaction. A case of secondary neurosyphilis gave a definite lilac colour. Other forms of syphilis, as exemplified by cerebral syphilis, gave negative results.

The only other fluid with a positive result was from a patient suffering from familial cerebro-macular disease. The intensity of the lilac colour was very weak.

It is of interest to look at the results in the other cases as, on examining the table, reports will be found upon fluids which showed isolated abnormalities. For instance, a fluid from a case of brain tumour, yellow in colour, gave a negative result. Again, several fluids containing a marked increase of the total protein content, as high as 1.0 per cent., failed to give a positive acetic anhydride reaction. It will also be noticed that positive Nonne-Apelt and Pandy reactions are possible with a negative reaction. Lastly, it is evident that changes in the Lange reaction are not associated with the reaction under discussion.

CONCLUSIONS.

The examination of this series of fluids clearly shows that the reaction cannot be associated with any single one of the usual syphilitic reactions, or with any known combination of these. It is possible to get a negative acetic anhydride reaction where there is an increase in cells and globulin, a paretic type of Lange curve, and a positive Wassermann reaction. Accordingly, it is only fair to assume that the 'causal factor' of the reaction must be some other substance in the fluid than that producing the other reactions. The suggestion that it may be due to an increase in the cholesterol content of the fluid appears a possible explanation.

We have found that a positive reaction occurs in practically every case of dementia paralytica, only one proving negative in our series; that a positive reaction may occur in secondary neurosyphilis; and that cases of cerebral degeneration may give a weak reaction.

REFERENCES.

- ¹ BOLTZ, *Amer. Jour. Psychiat.*, 1923, iii, 111.
- ² GROSSMAN, *Jour. of Ment. Sci.*, 1925, lxxi, 439.
- ³ HARRIS, *Brit. Med. Jour.*, 1926, i, 136.
- ⁴ STEEL and NICOLE, *Brit. Jour. Ven. Dis.*, 1926, ii, 243.