THE SODIUM CHLORIDE CRYSTALLIZATION TEST AND ITS RELATION TO THE BLOOD-C.S.F. BARRIER.

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[Received March 14, 1942.]

In 1936 Tomesco (1) and his co-workers described changes in the crystallization of sodium chloride produced by the addition of small amounts of cerebrospinal fluid to 0·8 per cent. saline. A drop of ·8 per cent. NaCl solution dried on a microscope slide at 75° C. produces a thin circumference of crystals surrounding an empty space. If normal C.S.F. is added in proportions of 1 C.S.F. to 20, 40 or 60 parts saline the central empty space is replaced by a thin translucent film of crystals, which in the higher dilutions begins to show lacunae. In a dilution of 1 in 120 this translucent film is reduced to a narrow strip along the inner margin of the circumference. Microscopically the translucent area is found to consist of concentric circles, feather- or spear-shaped crystals, parallel lines, needles, etc., well organized in the low dilutions, but not organized in a dilution of 1 in 120. This is regarded as the normal crystal picture.

The full-blown pathological reaction as described by Tomesco was obtained in a variety of neurological disorders, but particularly in fresh untreated cases of general paresis. In this reaction the lacunae do not begin to appear until very high dilutions are reached. In the proportion of 1 part C.S.F. to 120 saline the central area is still completely covered by a translucent film, which microscopically shows good organization into a variety of patterns. The latter are much finer in appearance than in the normal picture with low dilutions, and consist of concentric circles, liver lobule appearance, rosettes, feather- and spear-shaped crystals, radiations from a central crystal, etc.

Between these two pictures there are gradations which Tomesco and his co-workers termed " mixed " types. It seems probable that there is a gradual transition from the normal to the full pathological type, depending on the degree of abnormality of the fluid, and in our opinion the " mixed " pictures should be regarded as pathological reactions of greater or lesser intensity. In order to standardize the test as far as possible we have only used the dilution of 1 part C.S.F. to 120 saline. With this strength none but the most pathological fluids, such as those of untreated general paretics, give a picture in which the whole of the interior is covered by a film of crystals. We have designated this picture the " +++ " type. The " ++ " type, corresponding to Tomesco's " intermediate pathological," is characterized by empty spaces appearing in the central translucent stratum, the latter showing good organization. In the " + " picture (Tomesco's intermediate normal) the translucent film is further reduced to form a layer along the inner side of the circumference with poor organization.

The crystalline circumference also shows fairly characteristic features in the normal and three pathological types. These may be summarized as follows :

Normal pattern.—The circumference consists of a single layer of large cubical crystals with an irregular and amorphous outer margin. Here and there one sees a tendency to formation of three rudimentary layers.

" + " *type.*—More distinct differentiation into three layers with a space between each. Outer margin still irregular and rough.

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" ++ " *type*.—Definite arrangement into three layers, separated by two spaces, each layer being of more regular and smooth conformation. In places layers fuse together and become indistinct. Outer margin somewhat rough and amorphous.

" +++ " *type*.—Well-defined separation into three layers throughout whole circumference. Outer margin smooth and regular.

Technique of the Crystallization Test.

Apparatus.

1. The best microscopic slides. These must be chemically cleaned in concentrated acid bichromate, washed well with tap water, distilled water, and double distilled water, and then preserved in alcohol. When the slides are required they must be wiped with a fluff-free towel and afterwards polished with a piece of chamois leather. They are then placed in the oven at 75° C. for ten minutes to be certain that they are completely free from moisture. The slides should only be handled by their edges.

2. Six 5 by $\frac{1}{2}$ in. test tubes graduated at 6 c.c. These can easily be graduated in the laboratory.

3. Several 0·2 c.c. pipettes graduated in 0·001 c.c.

4. 0·8 per cent. sodium chloride solution. Only the purest sodium chloride should be used, and dissolved in fresh dust-free doubly distilled water.

Technique.

The C.S.F. is centrifuged, and 0·05 c.c. are pipetted into the graduated test tubes containing the appropriate amounts of saline. This is mixed well, and 0·02 c.c drops from each tube placed on the prepared slides which are lying on a tray. The tray is placed in a constant temperature hot-air oven at 75° for ten minutes, when the drops of saline-C.S.F. will be completely evaporated. The drops are examined macro- and microscopically.

Case Material.

Tomesco and his co-workers have described their results in 200 cases of mental disease. For details of his findings his paper should be consulted ; it is sufficient to point out that a small number of cases of constitutional psychoses like schizophrenia, epilepsy, manic-depressive insanity, gave pathological patterns. In untreated general paretics the picture was strongly pathological (" +++ ") in 100 per cent. of cases. Normal patterns were observed by them in all non-psychotic or non-neurological disorders. Finkelman (2) (1938), using Tomesco's technique, investigated the fluids from 68 psychotics (general paresis, epilepsy and schizophrenia) and found similar results. He further examined the fluids from 71 cases with general medical and surgical diseases, but without involvement of the nervous system, and found in every case a normal crystal pattern.

The present work is based on the examination of cerebrospinal fluids from 77 mixed cases, the bulk of which were selected because of the presence, actual or suspected, of organic, toxic or infective involvement of the central nervous system, however slight. Our object has been to see whether the views of Tomesco and his associates could be substantiated, and if possible to correlate the crystal picture with other findings in the fluid. In the subjoined table are enumerated the results of the crystal picture, total protein in mgm. per cent., globulin, colloidal gold tests, and the bromide distribution ratio between blood and C.S.F. The latter was carried out according to the method of Walther (3), i.e., the patient is given ·01 gm. NaBr per lb. body weight thrice daily for five days and on the sixth day blood and C.S.F. are withdrawn. The deproteinized specimens are then treated with a $\frac{1}{2}$ per cent. gold chloride solution and matched in a colorimeter. The normal ratio of bromide between blood and C.S.F. ranges from 2·8 to 3·3. This test has been used largely to determine the permeability of the blood-C.S.F. barrier (choroid plexus, meningeal vessels), and values below 2·8 are regarded as pathological, indicating increased permeability. Measurements of the surface tension of the fluid were also carried out in 42 cases by means of Traube's stalagmometer, but we found the experimental error so great that it was almost impossible to obtain consistent results on repeated examinations of the same fluid. For accurate results a du Nouy's tensiometer is required.

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For the estimation of protein Mestrezat's diaphanometric method was used. It consists of coagulating the protein with trichloracetic acid and boiling. The resulting turbidity is compared with that of a known series of standard tubes. For globulin the Pandy and Nonne-Apelt tests were used. A positive Pandy and negative Nonne-Apelt was interpreted as a ± result.

TABLE I.

Case No.	Sex and age.	Crystal picture.	Protein in mgm.%	Globulin tests.	Lange.	Br. D.R.	Diagnosis, etc.
1	M. 54	Normal	20	Neg.	00110 00000	2·83	General paresis (treated).
2	F. 32	N.	20	,,	11000 00000	3·1	Epilepsy.
3	M. 42	N.	22	,,	00000 00000	3·19	General paresis (treated).
4	F. 62	+	22	,,	11000 00000	2·84	Manic-depressive. General but no retinal arteriosclerosis.
5	M. 37	+	35	±	01232 20000	2·71	Chronic epidemic encephalitis.
6	F. 34	N.	15	Neg.	11000 00000	3·0	Catatonia.
7	F. 38	N.	20	,,	11000 00000	2·95	General paresis (treated).
8	M. 47	N.	20	,,	00110 00000	2·86	,, ,, ,,
9	M. 69	N.	25	,,	01110 00000	3·2	Melancholia, with moderate hypertension.
10	M. 44	+++	35	+	55432 21000	2·72	General paresis (treated).
11	F. 66	N.	15	Neg.	10000 00000	2·85	Secondary dementia.
12	M. 52	+	20	,,	11100 00000	3·01	Oligophrenia, with arteriosclerosis and hypertension.
13	F. 66	N.	20	,,	11000 00000	2·86	Senile dementia; hypertension. Retinal arteries normal.
14	M. 39	N.	20	,,	12332 10000	2·85	General paresis (treated).
15	M. 54	+++	60	+	55432 11100	2·0	Cerebral tumour.
16	F. 61	+	15	Neg.	11000 00000	2·85	Arteriosclerotic psychosis; marked retinal arteriosclerosis.
17	M. 59	N.	20	,,	12210 00000	2·83	General paresis (treated).
18	M. 35	+++	35	+	55432 10000	2·62	,, ,,
19	F. 60	+++	30	Neg.	00110 00000	..	Recurrent mania; C.S.F. removed during narcosis.
20	F. 60	+	20	,,	11000	2·81	Arteriosclerotic psychosis; retinal arteriosclerosis present.
21	M. 57	+	25	,,	11000 00000	2·83	Secondary dementia.
22	F. 41	N.	20	,,	11000 00000	3·05	Catatonia.
23	M. 55	++	25	±	12320 00000	2·74	General paresis (treated).
24	M. 63	+	25	Neg.	10000 00000	3·06	Epilepsy with general and early retinal arteriosclerosis.
25	M. 72	++	20	,,	11000 00000	2·79	Melancholia. Advanced genera and retinal arteriosclerosis.
26	F. 60	N.	15	,,	00000 00000	3·1	Paraphrenia.
27	F. 51	N.	25	±	21100 00000	3·3	Catatonia; weak positive Kahn in blood.
28	M. 65	+	25	Neg.	00000 00000	3·0	Melancholia; general and retina arteriosclerosis.
29	M. 57	N.	20	,,	11000 00000	3·2	Melancholia. Arteries normal.

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TABLE I (*continued*).

Case No.	Sex and age.	Crystal picture.	Protein in mgm.%	Globulin tests.	Lange.	Br. D.R.	Diagnosis, etc.
30	M. 37	N.	20	,,	11000 00000	2·85	Oligophrenia.
31	M. 51	N.	25	,,	21100 00000	..	Confusional psychosis.
32	F. 60	N.	20	,,	11000 00000	3·1	Epilepsy.
33	F. 40	++	30	±	21110 00000	2·73	Confusional psychosis; general and retinal arteriosclerosis present.
34	M. 45	N.	22	Neg.	00000 00000	2·96	Chronic epidemic encephalitis.
35	M. 52	N.	28	,,	11000 00000	2·9	Amnestic syndrome. Dyspituitarism.
36	F. 72	N.	20	,,	11100 00000	2·84	Senile dementia. Slight retinal arteriosclerosis. (?)
37	F. 56	+	20	,,	11100 00000	2·84	Alzheimer's disease with arteriosclerosis.
38	M. 46	+++	150	+	55555 54321	..	General paresis.
39	M. 42	N.	15	Neg.	11000 00000	3·32	Schizophrenia.
40	M. 63	N.	15	,,	00000 00000	3·4	,,
41	F. 63	++	25	±	21100 00000	2·67	Alcoholic psychosis (confusional syndrome).
42	M. 67	+	25	Neg.	11000 00000	3·1	Melancholia with general and retinal arteriosclerosis.
43	F. 36	+++	60	+	55555 54321	..	General paresis.
44	M. 69	N.	20	Neg.	00000 00000	3·1	Senile dementia.
45	M. 50	N.	25	,,	01221 00000	2·92	Melancholia.
46	F. 53	N.	15	,,	00110 00000	3·0	Involutional melancholia; with some general and retinal arteriosclerosis.
47	M. 23	N.	20	,,	00000 00000	3·3	Schizophrenia.
48	F. 34	N.	20	,,	11100 00000	2·78	Chronic epidemic encephalitis.
49	M. 47	+	25	,,	11100 00000	2·84	Arteriosclerotic psychosis; general and retinal arteriosclerosis.
50	M. 42	N.	30	,,	00111 00000	2·90	General paresis (treated).
51	M. 54	N.	20	,,	00000 00000	2·98	Secondary dementia.
52	M. 62	++	30	±	11110 00000	2·79	Epilepsy with arteriosclerosis.
53	M. 67	++	65	+	12332 10000	2·76	General paresis.
54	F. 69	+	25	Neg.	11000 00000	2·78	Senile dementia with general and marked retinal arteriosclerosis.
55	M. 49	N.	15	,,	00000 00000	..	Manic-depressive psychosis.
56	M. 46	+	25	±	01110 00000	2·84	Chronic epidemic encephalitis.
57	M. 34	+	25	Neg.	00000 00000	2·81	,, ,, ,,
58	M. 50	+++	168	+	42100 00121	..	Cerebral tumour.
59	F. 57	N.	20	Neg.	11000 00000	3·2	Involutional melancholia.
60	M. 55	N.	15	,,	00000 00000	2·92	Manic-depressive with angioneurotic oedema.

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TABLE I (*continued*).

Case No.	Sex and age.	Crystal picture.	Protein in mgm.%.	Globulin tests.	Lange.	Br. D.R.	Diagnosis, etc.
61	F. 44	+++	80	+	55443 21100	2·62	General paresis.
62	M. 47	N.	25	±	01110 00000	3·0	Tabo-paresis (treated).
63	M. 48	++	25	±	11000 00000	2·81	Arteriosclerotic psychosis; extreme general and retinal arteriosclerosis.
64	M. 57	++	30	±	32110 00000	2·65	General paresis (treated).
65	M. 36	N.	25	Neg.	10000 00000	..	Imbecility and cerebral angioma.
66	M. 54	+++	60	+	55555 54321	..	General paresis.
67	M. 56	+	35	±	44332 10000	2·68	„ „ (treated).
68	F. 38	N.	15	Neg.	00000 00000	3·4	Catatonia.
69	M. 44	+++	50	+	55555 43210	..	General paresis.
70	M. 53	+	35	±	22110 00000	2·78	„ „ (treated).
71	M. 35	+	20	Neg.	11000 00000	2·82	Epilepsy.
72	M. 55	++	35	±	22110 00000	2·79	Alcoholic psychosis with arteriosclerosis.
73	F. 44	N.	25	±	21100 00000	2·92	Paraphrenia.
74	M. 42	+	40	±	11111 10000	2·81	Cerebral syphilis.
75	M. 46	+++	45	+	01232 20000	2·43	General paresis.
76	M. 48	N.	..	..	..	2·86	„ „ (treated).
77	M. 41	+	..	..	..	2·78	„ „ „
				Non-psychotic patients with General Diseases.			
		N.	15	Neg.	00000 00000	..	Carcinoma of stomach.
		N.	15	„	11000 00000	..	Duodenal ulcer.
		N.	15	„	11000 00000	..	Pernicious anaemia.
		N.	25	„	00000 00000	..	Nephritis with uraemia.

Of the 77 fluids examined 39 gave a pathological crystal pattern, 19 a " + " type, 9 " ++," and 11 " +++." It will be seen that in practically every instance where the crystal pattern is pathological, evidence of cerebral organic involvement, severe, moderate or slight, is to be found. The involvement may be infective, toxic, arteriopathic or neoplastic. In all patients with clinical signs of arteriosclerosis the fundus oculi has been examined by the visiting ophthalmologist for the presence or absence of retinal arteriosclerosis. If the presence of arteriopathic changes in the retinal arteries is indicative of cerebral atheroma, then it must be inferred that the earliest demonstrable pathological change in the C.S.F. to be found in this disease is abnormality in the crystal picture, more particularly of the " one plus " type. A glance at the table reveals that the protein and globulin reactions are frequently normal and negative with a " + " picture; even a " two-plus " pattern may occur with normal protein figures. Table II shows the protein values in mgm. per cent. in relation to the various crystal patterns found, together with the mean values. In Table III an analysis of these figures along with the significance of the mean values is given. It is here evident that the difference of means between series I and II is not significant, as the probability is greater than 1 in 20 that the difference is due to random error. With the more pathological fluids the differences of means become significant, as one would expect. A similar

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TABLE II.—*C.S.F. Protein in mgm. per cent.*

Crystal picture:— Normal.	+	++	+++
20	22	25	35
20	35	20	60
20	20	30	35
15	15	25	30
20	20	30	150
20	25	65	60
25	25	25	168
15	25	30	80
20	20	35	60
20	25		50
20	25		45
20	25		
15	25		
25	35		
20	35		
20	20		
25	40		
20	20		
22	25		
28			
20			
15			
15			
20			
Mean 20·27	25·37	31·67	70·27

TABLE III.—*Analysis.*

	Series. I.	II.	III.	IV.
Crystal picture	Normal	+	++	+++
Mean C.S.F. protein mgm. per cent.	20·27	25·37	31·67	70·27
Standard error of mean	±1·38	±1·5	±4·34	±13·82

Significance of Mean Values of the C.S.F. Protein in mgm. per cent.

	Series compared. I & II.	I & III.	I & IV.	II & III.	III & IV.
Difference of means	5·1	11·4	50·0	6·3	38·6
Probability (P) that difference due to random error	P>·05	·01<P<·05	P<·001	·001<P<·01	P<·001
Significant	No	Yes	Yes	Yes	Yes

relationship exists between " plus one " pictures and the globulin reactions. In 13 fluids out of 18 in which the test was carried out the reaction was negative, and in the remaining 5 only ±. These findings would seem to indicate that in non-syphilitic organic cerebropathy the crystallization test is far more sensitive than any of the standard tests used with cerebrospinal fluid.

Of considerable interest too is the comparison between the crystal picture and the bromide ratio between blood and C.S.F. The results are shown in Table IV. Unfortunately it was not found possible to carry out the permeability test in a number of patients on account of resistiveness, but we believe there is sufficient evidence to show that the distribution ratio shows a definite downward trend with increasing abnormality of the crystal picture. This view appears to be confirmed by the statistical analysis set out in Table V, where the difference of means between Series II and III is the only one not significant.

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TABLE IV.—*Bromide Distribution Ratio between Blood and C.S.F.*

Crystal picture:

Normals.		+	++	+++
3·0	3·1	2·68	2·67	2·62
3·4	3·1	2·78	2·73	2·0
2·83	2·92	2·82	2·79	2·72
3·1	3·0	2·84	2·74	2·62
3·19	3·3	2·78	2·79	2·43
3·0	2·78	2·84	2·76	
2·95	2·90	2·81	2·61	
2·86	2·98	2·83	2·81	
3·2	3·2	3·06	2·79	
2·86	2·92	3·0		
2·85	2·85	2·84		
2·83	2·92	2·84		
3·05	3·1	2·71		
3·1	2·86	3·0		
3·3		2·85		
3·2		2·81		
2·85		2·81		
3·1		2·78		
2·96				
2·9				
2·84				
3·32				
3·4				
Mean value	3·03	2·81	2·74	2·48

TABLE V.—*Analysis.*

	Crystallization test.			
	Normal.	+	++	+++.
Number of cases	37	18	9	5
Mean bromide distribution ratio	3·03	2·81	2·74	2·48
Standard deviation	±0·172	±0·097	±0·062	±0·257
Standard error of mean	±0·003	±0·024	±0·022	±0·128

Significance of Mean Values of the Bromide Distribution Ratio.

	Series compared.			
	I & II. Normal. +.	II & III. +. ++.	III & IV. ++. +++.	II & IV. +. +++.
Difference of means	0·22	0·07	0·26	0·33
Standard error of difference	0·043	0·037	0·115	0·089
Probability that difference due to random error (by " t " method of student)	1 in 1000	1 in 14	1 in 25	1 in 500
Probability by " Z " method of R. A. Fisher	0·001<P<0·01	P>0·05	P<0·001	0·001<P<0·01

DISCUSSION.

The physico-chemical mechanism by which changes in sodium chloride crystallization is brought about through the addition of cerebrospinal fluid is a matter which must be solved by the research chemist. On the basis of the foregoing clinical investigation, however, the following facts emerge: (1) That a definite correlation exists between an increasing abnormality of the crystal pattern and an increasing permeability of the blood-cerebrospinal fluid barrier (or a decreasing distribution ratio); and (2) that with the more pathological patterns (i.e. the " two-plus " and " three-plus " types) there is a corresponding increase in the total

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protein values of the fluid. It seems probable that a qualitative and quantitative change in the C.S.F. protein picture is responsible for the pathological crystal patterns, but that with the slighter degrees of crystallization abnormality (our " + " type), the quantitative change in protein is so small that it cannot be estimated by the method employed in the present investigation. Tomesco ascribed the phenomenon to alteration in surface tension brought about by increased amounts of albumin and globulin, and Semenschenko and Shikhobalova (4) have demonstrated that the addition of surface active salts to a solution of sodium nitrate produced considerable effect, causing a greater degree of dispersion of the crystals than that of pure sodium nitrate. The sequence of events may therefore well be as follows: organic cerebral change→changes in the blood-C.S.F. barrier→heightened permeability→qualitative and quantitative alteration (i.e. increase) of C.S.F. protein, causing lowering of surface tension, which in turn would be responsible for the abnormality of the crystal picture.

Summary.

1. The influence of a minute quantity of C.S.F. on the crystallization of a ·8 per cent. sodium chloride solution has been studied.

2. On the basis of the examination of fluids from 77 mixed cases of mental disorder we are able to confirm the view of Tomesco and his associates that the crystallization test is more sensitive than any of the other classical tests in all the non-syphilitic psychoses investigated, where arteriopathic, toxic or infective changes of the brain were present. In cerebral arteriosclerosis it is usually the only abnormality to be detected in the fluid.

3. A positive correlation was found to exist between the degree of pathological change in the crystal picture and the lowering of the bromide distribution ratio between blood and C.S.F.

4. Alterations in the protein picture, both qualitative and quantitative, occur with the more pathological crystal patterns, but with a mild degree of abnormality such as the " one-plus " picture no significant alterations could be demonstrated.

5. A tentative explanation of the mechanisms involved has been put forward.

Our thanks are due to Lt.-Col. Graeme Talbot, R.A.M.C., visiting ophthalmologist, for reporting on the fundus oculi in a number of cases, to Dr. Tudor Jones for assistance in the preparation of statistical analyses, and to Mr. T. C. Hall, laboratory technician, for much painstaking and accurate work.

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