BIOLOGICAL AND SEROLOGICAL METHODS OF DIAGNOSIS IN EPILEPSY.*

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PATHOLOGO-HUMORAL methods of research which have been pursued in epileptic patients may be useful in three different respects : (1) The results and obtained relations inform us of the beginning of the disease and of the way the epileptic attacks occur. (2) They may be used for causal treatment. (3) They offer a convenient method, on a pathologo-humoral basis, for the diagnosis of epilepsy.

In all three respects these humoral and pathological researches have given valuable results. Researches on the phases of epilepsy have also led to essential conclusions. They show the modifications which appear in epilepsy during periods where no symptoms are to be observed, as well as in relation to the attacks.

The study of metabolism shows that in the processes of oxidation there appears a decrease compared with the normal. Experiments with carbohydrates and albumin show a relative decrease of humoral activity and of the production of carbonic acid. The azotic exchanges prove a modification related to the epileptic attack. Before the paroxysms one finds the smallest values of nitrogen, and after the attack it rises again. During the attacks one observes nitrogen retention, which Allers¹ attributes to circulating albumin. As for exogenous purins, the study of metabolism (as Rohde²² has shown) proves a slower combustion of these. One may observe a diminution in the rate of excretion of urinary acids. Some observers mention also a toxic condition of the nucleoproteins.

The alterations of the elements which occur in epilepsy naturally react upon the state of the body humours. Chemical, biological and morphological blood analyses also show alteration, which Di Gaspero and De Crinis⁹ have first demonstrated to occur. *If we sum up the humoropathological characteristics of the blood serum, we see that the albumin quotient of the serum in epileptics offers greater fluctuations than in normal persons.* Before the attacks the albumin content goes rapidly up, reaching its highest point immediately before them ; afterwards it falls suddenly. The rise and fall of the tension of blood shows an evident parallelism with the oscillations of the albumin. The time of coagulation may also fluctuate, and, in particular, be longer before the attack. Di Gaspero has also noticed leucopenia before the attack, and, on the contrary, leucocytosis during the attack. Further, one observes a rise in hæmoglobin

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and in viscosity as well as a decrease in the specific gravity of blood during the epileptic attack.

Bisgaard⁴ observed in 30 genuine epileptics a decrease of the percentage of H-ion concentration and also of NH_3 excretion, whereas he failed to find a similar decrease in 150 normal people. Disturbances of that kind are well known in tetany and are disclosed by the dosage of parathyroids. From this point of view Bisgaard considers that tetany and epilepsy are due to functional alterations of the endocrine glands. Bolten⁵ also considers epilepsy as due to insufficiency of the thyroid and parathyroid glands. Pfeiffer¹⁹ and De Crinis have also found a rise of the antitryptic ferments in blood serum. According to Bruehl⁷ the proportion of creatines shows diminution, and, with the attack, higher values.

Among lipid substances the quota of cholesterin in serum offers fluctuations during the attack similar to those of albumin. These facts deserve particular notice as the central nervous system is the chief storehouse of the lipoids, especially cholesterin; and in epileptic attacks alterations of the central nervous system are to be put in the first rank.

Cuneo⁸ has observed that the serum contains toxic albuminoses, especially before the attack. Many authors consider the pathological basis of epilepsy as the effect of these toxic substances contained in blood. Pfeiffer¹⁹ has also shown the rise of parenteral albumin as a cause of epilepsy. For this reason, it may be assumed, the disease belongs to the class of toxic anaphylaxis.

Humoral alterations of the serum, observed during the attacks, occur indeed by themselves, without any attack. We term these the 'serologic equivalents' of the epileptic attack. It is very important from a practical standpoint to utilise humoral and pathological methods for the diagnosis of the disease.

This proof of the presence in the epileptic organism of toxic substances may be used for the diagnosis of the disease from a biological point of view. Of the chemical constitution of those bodies, acting toxically and appearing in body fluids after the epileptic attacks, we know nothing for certain. Some authors from experiments upon animals call in question the toxicity of the urine after the epileptic attack. Loewe¹⁶ asserts strongly a relation of toxic elements of the serum to a substance in the urine which cannot dialyse and contains nitrogen. This substance, which he calls 'pesotexine,' is excreted after the attack in great quantity in the urine and can produce, according to his experiments, epileptic seizures in animals. These experiments do not however show a sufficiently coherent result. Examinations of the urine evacuated by catheterization and inoculation of epileptics' urine in animals are of no help for the diagnosis of epilepsy; they have no significance since toxicity of urine is observed in many other psychoses. And furthermore those methods do not give results during the periods free from attacks, when it should be most desirable to make a diagnosis.

The explanation of urinary toxicity after the paroxysms is differently accounted for. Noervig¹⁷ asserts that according to his experiments the epileptic organism presents disorders in the regulation of the H-ion concentration in urine. De Crinis points out that the central nervous system in the epileptic loses the power to regulate the 'acid-base equilibrium.'

There is far less discrepancy, if instead of inoculation by urine, animals are inoculated with epileptics' blood serum. During last year I had occasion in our clinic to pursue such experiments. We have worked out a biological method principally for the serological diagnosis of dementia præcox.

On the basis of the technique published in 1911 by Benedek and Deak³, our aim was to study the degree of formation of immun-hæmolysin in rabbits which had been inoculated with blood serum of patients suffering from dementia præcox, general paralysis, epilepsy and some other nervous or mental diseases, and also the blood serum of healthy persons. So far we have three series of experiments and have inoculated 36 rabbits in 136 cases with blood serum of different patients, using either the venous or the intraperitoneal route. The number is explained by the fact that we have many times taken blood from the same person and used it for inoculation.

Our researches, principally the inoculation of blood serum of patients suffering from dementia præcox, led us to interesting results.

In our experiments we have evaluated the content of immun-hæmolysin of the blood serum of rabbits, as follows. One part of the immune serum was made inactive by leaving it 1½ hours in a water bath at 56° C. Each rabbit serum was used in that way to obtain two distinct series: active and inactive. We filled up 1 c.c. with an 0.85 per cent. NaCl solution, the dose of immune serum increasing from 0.1 or 0.2 to 0.5. Afterwards we added in the active series in each separate test tube another c.c. of the 0.85 per cent NaCl solution. In the active series we took only 1 c.c. of the 0.85 per cent. NaCl solution and ½ c.c. 10 per cent. fresh complement. In the two series we placed in each test tube of each group (in which were 5 tubes) ½ c.c. for every separate test tube of a 5 per cent. emulsion of washed red blood corpuscles—to the first group, of normal persons (or in some cases of epileptics); to the second, of patients suffering from dementia præcox; and to the third group, of patients suffering from general paralysis.

We obtained the 5 per cent. red blood corpuscles under the usual conditions, from fresh blood taken by venous puncture and defibrinated after careful washing with physiological NaCl solution. We kept the glass tubes of all groups in the incubator at 37° C. during two hours, and the result was usually read next morning when the series had stood for 10—12 hours at room temperature.

The normal content of hæmolysins in blood serum of rabbits as proved by our experiments is very small, and capable only in the highest titre of producing weak hæmolysis. The serum of the various rabbits shows only small deviation with reference to normal hæmolysin content. In spite of that

we have evaluated in each case the hæmolysin content before the injections, in order to compare this afterwards with the titre of the immun-hæmolysin attained after repeated injections. The technique in those inoculated animals was in some details variable. According to our researches the best method is to inoculate the rabbits intravenously with the serum of patients, performing the injection at intervals of four to five days. We always repeat it three times with doses of 3—4 c.c. The fifth day after the last injection, we take the blood of the animal by heart puncture and we again examine the immune serum with the different emulsions of red blood corpuscles in each of the described hæmolytic systems.

In all three groups of our experiments constant differences showed themselves between the results—according to the formation of immun-hæmolysin—of the inoculation on the one hand of serum from cases of dementia præcox, and on the other of serum from general paralytics or other mental patients, or, lastly, of healthy subjects.

For dementia præcox it may finally be accepted as a specific and special humoral characteristic that the inoculation of serum of patients suffering from that disease gives the highest titre of immun-hæmolysin formation in inoculated animals. We cannot describe that occurrence by a better name than 'positive pleohæmolytic reaction.'

During the 'pleohæmolytic' researches we have used as a control method the blood serum extracted from 10 inoculated epileptics. We have injected in the ear-vein of rabbits 3—4 c.c. of blood serum. In six cases the animals were seized with tonic and clonic attacks from a quarter to one minute after the injection and three died during the seizure. The other inoculation of epileptic blood serum in animals was always intraperitoneal. In this way we did not obtain proof that the toxic blood serum could cause the attack, but only that after a series of injections the body-weight would notably decrease. Similar phenomena were observed in 15 cases published in 1921 by Pagniez, Mouzon and Turpin¹⁸. They inoculated rabbits in the heart with 2—4 c.c. of blood serum of epileptics. In three cases they produced a seizure. If the blood serum is warmed for ten minutes at the temperature of 58° C. it loses its toxic power.

The above-mentioned biological methods (with blood serum inoculations) may also be applied to the diagnosis of uncertain cases. From another point of view Trevisano²⁴ has employed the inoculations of serum for this purpose. He utilised anaphylactic shock in his experiments to prove epileptic causation. He first inoculated guineapigs with blood serum of normal subjects and some days after with spinal fluid from the same. No anaphylactic symptoms were observed. The same experiments pursued with blood serum and spinal fluid of epileptics bring anaphylactic symptoms into evidence. The animals exhibited general trembling, seizures, and decrease of the body temperature; and in many cases death ensued. Identical effects appeared when first spinal

fluid and then blood serum were inoculated. The results of these inoculations do not seem to be influenced by the matter of whether spinal fluid and blood serum obtained immediately after the attack are used, or those of some time after.

Of the toxicity of epileptic spinal fluid we know nothing for certain. Kafka¹⁴ tried to solve the question by well-considered experiments, but reached the same conclusion. Experiments were also made on the cytological, biological and chemical character of the spinal fluid and fluctuation in its pressure. The results of various investigations on the spinal fluid spectrum (Eskuchen¹¹) or on the so-called 'humoral syndrome' in nervous and mental diseases have given very poor results for epilepsy. Our own researches, using epileptic spinal fluid collected during the attack as well as after it, did not demonstrate anything abnormal with the various methods of research already known. An increased spinal fluid pressure is usually found, especially during the attack. These observations had already been made by Redlich and Poetzel²¹ in 1919 and afterwards by Boveri⁶. We have observed it ourselves in one-third of all cases. Suboccipital puncture showed in general that in the sitting position the spinal fluid pressure in the cisterna magna was (even during the periods free from attacks) positive, contrary to the normal negative pressure. In these cases the spinal fluid on puncture drops out by itself, when the patient is sitting.

Mention should be made of the evidence furnished by Donath¹⁰ that the spinal fluid collected during the attack contains choline. Relying on this, he thinks that choline plays a part in the causation of the epileptic attack.

Notice also should be taken of the fact proved by Laures and Gascard¹⁵ that whereas the urea content of spinal fluid increases during the epileptic attack, it decreases during the hysterical seizure; and this may be used for differential diagnosis. Wittgenstein²⁴ has observed hyperglycorrachia immediately after the attack. Recently Jacobi¹³ has shown disorder of the 'interferometric rate' in the spinal fluid of epileptics, mostly an increase. Altenberger and Stern² assert that a secretion of the retrohypophysis is to be found in the spinal fluid. It was absent at the beginning of 79 cases of epilepsy, whereas they had found in 71 cases out of 80 of non-epileptics an average percentage of 0.00058 in the spinal fluid taken from the cisterna magna or of 0.00087 in that from the ventricles.

In clinical practice as regards the humoral-pathological diagnosis of epilepsy the above researches cannot be very useful as they depend upon difficult chemical analyses.

Hartmann and Di Gasparo¹² with methods used in clinical practice, found in epileptic cases a notable decrease of the albumin content of spinal fluid (·04—·06 per cent.) (Nissl-Esbach method). On the whole the chemical features of the spinal fluid in epileptics do not show anything certain when examined with methods already known. De Crinis stresses the fact that

“nothing characteristic is shown by the spinal fluid chemical standard in epileptics.”

In our clinical laboratory, out of 5,780 spinal fluid examinations since 1922 to the present day, 332 concerned cases of epilepsy. We have observed always an absolutely negative ‘spinal fluid spectrum.’ Among colloid reactions of greatest sensitivity, the normomastic and the shellac reactions, and also the bicoloured mastic reaction²³, have in some instances given very evident deviations.

We have described the whole humoral syndrome on the basis of the so-called ‘five reactions,’ summarizing our researches—numerous enough—upon epileptic spinal fluid. These include the Wassermann reaction of serum and spinal fluid, cytological analysis, globulin and colloid reactions. We have also compared the results of these various methods. Our results are shown in Table I. A slight pleocytosis in epileptic spinal fluid occurs only in a small percentage of cases. The globulin and goldsol reactions seldom exhibit departure from the normal, and that is little. The highest percentage of these mild deviations is shown by the bicoloured mastic reaction, in the form of slightly irregular curves. *For that reason this colloid reaction may be regarded as the most precise among them all.*

The numbers between parentheses in the Table refer to cases where a small quantity of blood in the fluid or a reaction due to intervention (air or lipiodol injection) has modified the findings, or where the examined epileptic exhibits a seropositive latent syphilis. The great value of the bicoloured mastic reaction in the range of the ‘5 reactions’ is shown also by the fact that in 16 cases we have found a feebly positive reaction proved by slight irregularity of curves in patients who showed also a seropositive latent syphilis.

After two years’ research we have in our laboratory in Debrecen worked out a new method for the well-known colloid reaction based on a quite different base: the China ink reaction of the spinal fluid. This technique is also useful for the examination of the spinal fluid in epileptics, as I will briefly show. The method is very simple and for that reason very useful in general practice. In three small test tubes we put 0.1 c.c. spinal fluid per tube and thereafter in the first one drop, in the second two drops and in the third three drops of $\frac{1}{2}$ per cent. oxalic acid. We add to each tube 1 c.c. of a 1 per cent. solution of the original solution of China ink (Günther Wagner’s original ‘Perltusch’). We prepare freshly this 1 per cent. solution by diluting the China ink with distilled water.

The dilution of the 1 per cent. China ink solution demands great attention, as the original solution adheres to the pipette and so an additional drop in the contents of the pipette might pass unnoticed. The required solution is prepared with China ink in small flasks and then the pipette is thoroughly rinsed with it.

The result of the reaction can be read after 15 to 20 minutes and also after centrifuging after for a half to one minute. We notice in some test tubes the China ink is completely precipitated and above the precipitate the solution is as clear as water. In other tubes there is no alteration to be noticed; or, in

the intermediary state of the colloid protection to the precipitation, some precipitate is found at the bottom of the tube, over which a pale black solution is visible.

Since the technique of the test and the reading of its results are quite different from those of other colloid reactions, it is not possible to record the results according to the degree of precipitation. We record the result of the China ink test in a new fashion. In the tubes which remain unchanged all the colloid is protected against a precipitating effect, and this we indicate by the sign **n**. When complete precipitation has occurred we put the sign **u**. The intermediary state between colloid protection and precipitation is indicated by —. We write near each other the result read in each tube. The result consequently is in normal spinal fluid as follows : **uun**.

It seems to us, however, practicable to tabulate the result of this test in figures as well. Whereas in normal spinal fluid the precipitates are observed in two test tubes, on the contrary in positive spinal fluid one notes colloid protection ; and for that reason we express the results by the ' colloid protecting index ' (C.P.I.). We obtained the C.P.I. as follows. In the first tube we mark the precipitation as —1, the colloid protection as +1. In the same way, in the second and third tubes are marked —2 and —3 (precipitation), and also +2 and +3 (protection). The intermediary reaction degree in each tube is noted with 0. Numbers so obtained are written in succession, and making a total—we get a series of figures which is called the ' colloid protecting index.' According to this the result is a negative spinal fluid is noted as follows : —1, —2, +3=0. In normal spinal fluid the C.P.I.=0.

In the laboratory of the neurological Clinic in Debrecen we have investigated this test in various diseases of the central nervous system. In cases of epilepsy, as may be seen from the Table, the C.P.I. deviates from the normal in a characteristic way. We usually obtained the following result : **nun**=1, —2, +3. This means that in epileptics we get mostly the result C.P.I.=2. In general we can say that the China ink test is positive in 87 per cent. of cases of epilepsy, which makes it decidedly superior to the other tests which have been described up to the present.

THE EXAMINATION OF SPINAL FLUIDS OF EPILEPTICS WITH DIFFERENT TESTS, AND COMPARISON OF THE RESULTS. (RESULTS IN PERCENTAGES.)

PLEOCYTOSIS.

Number of cases	Number of cells 0 in c.c.	Number of cells $\frac{1}{3}$ -1 in c.c.	Number of cells 1-2 in c.c.	Number of cells 0-2 in c.c.	Number of cells 3-5 in c.c. slight pleocytosis	Number of cells 5-20 in c.c.	Number of cells 20-50 in c.c. strong pleocytosis	Number of cells more than 50 in c.c. very strong pleocytosis
286	114 39.9	100 34.9	32 11.2	246 86.0	21 (+6) 7.3(+2.1)	6 (+2) 2.1(+1.0)	0(+2) 0(0.8)	0(+2) 0(0.8)

GLOBULIN REACTIONS.
(Pandy, Nonne-Apelt, Ross-Jones, Weichbrodt tests.)

Number of cases	All globulin tests are negative	Pandy + or Weichbrodt $\pm$ the others negative	All cases which can be taken as negative	Slight positivity of Pandy test; one or more globulin reactions +	Medium positivity of Pandy and ++ of one or more globulin reactions	Strong positivity of all globulin reactions
325	261 80.5	16 (+15) 4.9 (+4.6)	292 89.80	19 (+10) 5.9 (3.1)	0 (+2) 0 (0.6)	0 (+2) 0 (0.6)

GOLDSOL TEST.

Number of cases	Negative curve	Curve with a slight rise	Medium alteration above the third reaction degree	Curve of meningitic type
300	287 95.6	6 (+5) 2.0 (+1.8)	0 (+1) 0 (0.3)	0 (+1) 0 (0.3)

BICOLOURED MASTIX TEST.

Number of cases	Negative curve	Slight alteration without rise of curve	Slight rise of curve	Medium positivity above the third reaction degree	Curve of meningitic type
289	180 62.2	31 10.7	44 (+16) 15.2 (+5.6)	4 (+10) 1.4 (3.5)	0 (4) 0 (1.4)

SHELLAC TEST.

Number of cases	Negative results	$\pm$ results	+ results	++ or +++ results
176	146 82.9	7 (+3) 3.9 (1.7)	4 (+7) 2.4 (3.9)	0 (+9) 0 (5.2)

CHINA INK TEST. COLLOID PROTECTING INDEX C.P.I.

C.P.I.=0	C.P.I.=2	C.P.I.=4	C.P.I.=6	China ink test positive	China ink test negative
12.9	77.4	9.7	0	87.1	12.9

Wassermann reaction in spinal fluid negative in all cases (332). Wassermann reaction positive in the blood in 45 cases (13.6 per cent.) out of 332.

REFERENCES.

- ¹ ALLERS, *Zeits. f.d.g. Neurol. u. Psychiat.*, 1912, iv, 833.
- ² ALTENBERGER and STERN, *Zeits. f.d.g. Neurol. u. Psychiat.*, cxii, 5 Heft.
- ³ BENEDEK and DEAK, *Arch. f. Psychiat.*, 1911, li.
- ⁴ BISGAARD, *Zentralbl. f.d.g. Neurol. u. Psychiat.*, 1922, xxix.
- ⁵ BOLTEN, *Deut. Zeits. f. Nervenheilk.*, 1917, vii, 160.
- ⁶ BOVERI, *C. R. Soc. de biol.*, lxx.
- ⁷ BRUEHL, *Zeits. f.d.g. Neurol. u. Psychiat.*, 1923, lxxxiv, 642.
- ⁸ CUNEO, *Riv. sper. d. freniat.*, 1922, xlv.
- ⁹ DE CRINIS, *Zeits. f.d.g. Neurol. u. Psychiat.*, 1925, xcix, 718.
- ¹⁰ DONATH, *Orvosi Hetilap*, 1905.
- ¹¹ ESKUCHEN, *Lumbalpunktion*, 1918.
- ¹² HARTMANN and DI GASPERO, *Lewandowsky's Handbuch d. Neurol.* Bd. v, 1912.
- ¹³ JACOBI, *Zeits. f.d.g. Neurol. u. Psychiat.*, 1923, lxxxv.
- ¹⁴ KAFKA, *Aschaffenburg's Handbuch d. Psychiat.*, 1927.
- ¹⁵ LAURES and GASCARD, *Presse méd.*, 1920.
- ¹⁶ LOEWE, *Zeits. f.d.g. Neurol. u. Psychiat.*, 1911, 73.
- ¹⁷ NOERVIG, (ref.) *Zentralbl. f.d.g. Neurol. u. Psychiat.*, 1922, 401.
- ¹⁸ PAGNIEZ, MOUZON and TURPIN, *C. R. Soc. de biol.*, 1921, lxxxv.
- ¹⁹ PFEIFFER, *Mitteil. d. Ver. d. Arzt. in Steiermark*, No. 81.
- ²⁰ PIGHINI, *Zeits. f. physiol. Chem.*, 1919, lxi, 518.
- ²¹ REDLICH and POETZL, *Zeits. f.d.g. Neurol. u. Psychiat.*, iii, 715.
- ²² ROHDE, *Deut. Arch. f. klin. Med.*, 1908, xcv.
- ²³ THURZO, *Deut. Zeits. f. Nervenheilk.*, 1926, xci.
- ²⁴ TREVISANELLO, *Zentralbl. f. Bakteriol.*, 1913, lxiii, 163.
- ²⁵ WITTGENSTEIN, *Deut. med. Woch.*, 1928.