sättigt. HAVEN[25] hat letzthin mitgeteilt, daß Fettbestandteile aus dem Wirtsorganismus in die wachsende Krebsgeschwulst abwandern und in Form von Polyensäuren in die Phosphatidfraktion des malignen Gewebes eingebaut werden. Durch quantitative Ermittlung des lipoiden Energiepotentials und seine bilanzmäßige Koordinierung sind neue Erkenntnisse hinsichtlich der Energieversorgung zellphysiologisch lebenswichtiger Funktionsbereiche zu erwarten.

[24] H. J. DEUEL, l. c. [2], S. 353.
[25] F. L. HAVEN, W. R. BLOOR u. CH. RANDALL, Cancer Res. **11**, 619 [1951].

Biochemical Studies on Sera or Cerebrospinal Fluids of Neurologically Normal Persons and Patients with Multiple Sclerosis

By E. Kovács ** and J. Kovács

III. Phosphomonoesterases, their inhibitors or activators in sera and CSF's of MS cases *

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Phosphomonoesterase-Aktivitäten in Normalseren und in denen von M.S.-Kranken wurden gleichzeitig auf das Vorhandensein von Hemm- bzw. Aktivator-Effekten gegen gereinigte oder konzentrierte, natürliche Enzyme untersucht. Saure, resp. alkalische Phosphatase und 5-Nukleotidase wurde in Serumgroßpoolen von gesunden Menschen (über 600 Einzelseren beider Geschlechter, im Alter von 20 bis 65 Jahren) bestimmt, gleichzeitig auch die spezifischen Einflüsse der Seren auf gereinigte pflanzliche saure und tierische alkalische Phosphatase, bzw. die 5-Nukleotidase von lyophilisierten Schlangengiften. Die Großpoole beider Gruppen, getrennt nach Geschlechtern, zeigten einen ermäßigten Spiegel der gewöhnlichen Phosphomonoesterasen, die 5-Nukleotidase Aktivitäten wurden aber besser erhalten. Dieselben Seren aktivierten die 3 heterologen Phosphomonoesterasen und zwar vorwiegend die saure Phosphatase, viel geringer die alkalische Phosphatase und in unbedeutendem Umfang die spezifische Nukleotidase. Wurden die Poole beider Gruppen gemischt, so zeigten die Enzymwerte ein modifiziertes Verhalten, aber die Änderung des kinetischen Einflusses der Seren war noch deutlicher. Die Beschleunigung der Aktivität der heterologen sauren Phosphatase hat abgenommen; die alkalische Phosphatase wurde sehr stark aktiviert und die Wirkung auf Schlangengift 5-Nukleotidase zeigte nur eine geringe, unbedeutende Abnahme. Wurde ein weiterer Pool aus einigen normalen Seren bestehend (freiwilligen Spendern) unter ähnlichen experimentellen Bedingungen geprüft, so waren die Ergebnisse wiederum etwas verschieden.

Kleine Poole von Seren M.S.-Kranker waren dagegen bei Abwesenheit der sauren Phosphatase-Aktivität, durch niedrige 5-Nukleotidase und erhöhte alkalische Phosphatasenwerte charakterisiert. Diese Enzym-Aktivitäten änderten sich in den Poolen von M.S.-Seren nach der Nahrungsmittel-Aufnahme *anders* als in denen Gesunder. Nicht eingefrorene, frische Seren von chronischen M.S.-Kranken (Frauen mit einer Krankheitsdauer von über 10 Jahren) wurden *vor und nach* der Nahrungsaufnahme untersucht und Analogien bzw. Verschiedenheiten mit den oben erwähnten, nach Einfrieren untersuchten Proben festgestellt. Weitere Untersuchungen klärten einige Diskrepanzen und betonten die Wichtigkeit des einheitlichen methodischen Vorgehens. Die quantitative *Zunahme der 5-Nukleotidase-Hemmung nach Aufbewahrung in der Kälte wurde als typisch bei Seren M.S.-Kranker beschrieben.* Schließlich wurde eine ähnliche Hemmung oder Beschleunigung einiger Phosphomonoesterasen in den Liquoren von M.S.-Patienten beobachtet. Diese Eigenschaften *fehlten* in dem Großpool gesunder Liquors. Verschiedene Aspekte der Problematik wurden auf Grund eigener Erfahrungen oder Arbeitshypothesen über die Herkunft der beschriebenen *biochemisch-aktiven* Substanzen bei M.S.-Kranken untersucht und besprochen.

Previous contributions were concerned with the definition of normal controls[1] and the effect of normal sera on purified or concentrated natural enzymes in vitro[2]. As the result of this screening the collection of a large pool of specimens from neurologically normal male or female persons was decided (over 600 for each sex) with exclusion of age groups under 20 years, together with lipaemic, haemolytic, prenatal, icteric and Wassermann-positive sera. The results of phosphomonoesterase

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[1] E. Kovács, Z. Vitamin-, Hormon- u. Fermentforsch. [Wien] **10**, 116 [1959].

[2] E. Kovács, Z. Vitamin-, Hormon- u. Fermentforsch. [Wien] **10**, — [1959].

assays on this control group and MS specimens will be reported in the present publication. The common acid respectively alkaline phosphatase and the specific 5-nucleotidase were investigated in the sera, together with their *kinetical effects* on enzyme concentrates of various origin. Although work on enzymes in connection with demyelination is known [3, 3a] and many writers attribute an etiological role to abnormal activity of certain biocatalysts in the genesis of MS [3b], the joint assay of enzymes and their autologous inhibitors or activators [2] is a novel approach to the understanding of the biochemistry of this disease. The different behaviour of MS sera in many respects, as compared with the normal controls, is prompting us to publish this report, making the data accessible to others, especially to *clinical workers* interested in MS research.

Materials and Methods

The sampling or screening of the specimens and the way of collection of the normal material was described [1]. The samples were deepfrozen at $-25\,^{\circ}C$ as a rule, thawed before use and then stored at $4\,^{\circ}C$ till the completion of the assays (max. $8-10$ days). The majority of MS specimens was collected in a special clinic * for walking and easily transportable patients, or in hospitals for chronic cases ** and finally in the homes of a special small group of chronic patients. No other routine, than the general aseptic handling was adopted, unless not stated otherwise [1, 2].

The sera were tenfold diluted with bidestilled water, immediately before use for the enzyme assays, carried out as previously described [1, 2]. The techniques of SHINOWARA et al. [4] were used for *acid and alkaline phosphatases* (ph-ases) with Na-β-glycerophosphate substrate buffered to p_H 5 resp. p_H 10.9. For 5-nucleotidase (5-NT-ase) the method of REIS [5, 1] was employed. Occasionally the micro-method of HEPPEL et al. [6] and especially their recommendation of storing the muscle-adenylic acid substrate by deep-freezing was applied, but for the macro-technique we used veronal-acetate buffer as before [1, 2]. *Experiments with purified or concentrated heterologous enzymes* for the investigation of the kinetical effects of the specimens were carried out as before [2]. Purified wheat-germ *acid phosphatase* (ph-ase) and calf intestinal alkaline phosphatase * were dissolved (0.1 to 0.01%) in water and assayed with or without the addition of serum to the test-systems [2]. The alkaline phosphatase required magnesium activation. Different salts were tried, $MgSO_4$ being used generally in 0.020 M final concentration.

Lyophylized snake venoms ** (Naja-Naja and Russels viper venom) rich in 5-NT-ase were used in 0.1 to 0.01% concentrations. The water-dissolved enzyme materials were reasonably stable for $1-2$ weeks, the 5-NT-ase of the venom being inactivated the most rapidly [7].

Composition of the test-systems: The same final volumes were used as a rule, to obtain comparable values. For instance in a typical experiment 0.5 ml concentrated enzyme, 1.5 ml H_2O and 6 ml substrate were mixed. The enzyme material was brought up to 2 ml with water, thus the Enzyme : Substrate ratio was 2 : 6. When desired the water was partially replaced by the material to be tested for specific activity, such as serum, brain homogenate, etc. *Substrate and enzyme controls* were run in parallel and were similarly handled as the test-system. Incubation was usually 2 hours at $37\,^{\circ}C$ to allow sufficient time for interaction. The reaction was stopped by addition of 2 ml 30% ice-cold Trichloroacetic acid; for filtration acid extracted Whatman Nr. 42 paper was used. *Inorganic phosphatases* (IP) were measured by the colorimetric techniques of FISKE and SUBBAROW [8] and the *relative enzyme* activities expressed as increase in IP μg/ml of the reaction mixture over the kinetics of enzyme concentrates. The following symbols were adopted: a ϕ sign was used when the effect was additive (the sum of activities of serum and enzyme assayed separately *equalled* the values found with a system composed of both). On the other hand $>$ meant higher and $<$ lower values found, than calculated. The difference was expressed as % activity [2].

Results

Table I illustrates typical assay-results of phosphomonoesterases in various serum pools. Mean values of duplicate experiments are tabulated. The large normal groups exhibited *low acid and alkaline phase, but a relatively high 5-NT-ase* activity. When equal volumes of the samples of the two sex-groups were mixed and assayed, the enzyme values *increas-*

[3] E. W. HURST, Austral. J. exp. Biol. med. Sci. **20**, 297 [1942].
[3a] C. E. LUMSDEN, Brit. med. J. **1**, 1035 [1951].
* The courtesy of the Staff of the Toronto Western Hospital, Sunnybrook Hospital, Our Lady of Mercy Hospital and the Toronto Chapter of the MS Society of Canada is gratefully acknowledged.
** Courtesy of Dr. J. L. SILVERSIDES, Chief neurologist in the Toronto Western Hospital, Drs. J. and P. O'HARA, staff physicians in Our Lady of Mercy Hospital.
[4] Y. K. SHINOVARA, K. L. JONES and H. L. REINHARDT, J. biol. Chemistry **142**, 921 [1942].
[5] J. REIS, Enzymologia [Den Haag] **5**, 251 [1938/39].
[6] L. A. HEPPEL and R. J. HILLMOE, in: COLLOWICK and KAPLAN's Methods in Enzymology, Academic Press, Edit. 1955, Vol. 2, p. 546.
** Supplied by the Nutritional Biochemical Corporation, Cleveland, Ohio, USA.
[7] E. KOVÁCS, Z. Vitamin-, Hormon- u. Fermentforsch. [Wien], 1959, im Druck.
[8] C. H. FISKE and Y. F. SUBBAROW, J. biol. Chemistry **66**, 375 [1925].

ed (see for instance the alk. ph-ase). The small pools of *normal* volonteers did show higher alk. ph-ase but in general lower acid ph-ase and 5-NT-ase values than the precedent groups. The activity of acid ph-ase increased significantly, the 5-NT-ase was unchanged and the alk. ph-ase decreased *after meals.* The comparable small MS group did show an *absence* of acid ph-ase and 5-NT-ase in the diluted serum pool *before meal* but usually an *increase* in enzyme activities was found *after meal.*

Table II illustrates the effect of the normal and *MS serum pools* on purified acid resp. alkaline phosphatases and on unpurified 5-NT-ases of snake venoms. The large banks of normal male and female sera *activated* considerably the pure acid ph-ase, but the mixture of both sex groups was less active. There was only a low activating effect observed (with the separated pools) on alkaline ph-ase and no significant inhibition resulted with 5-NT-ase. However the mixed large pools activated strikingly the alk. ph-ase and did not influence the nucleotidase.

The activation of acid ph-ase by the small serum pool of normal persons was of much lower order, than the one observed with the large pools. The activation of *alk. ph-ase* however was much higher in the small pool. There was a definit inhibition found in the 5-NT-ase assays before meal. All these specific effects diminished *after meals*; there was a not significant difference with acid ph-ase and 5-NT-ase, but a considerable one with alkaline ph-ase, where the overall change was 65%.

The effect on acid ph-ase of the *MS serum* pools was not significant before meals. The activation of alkaline ph-ase was *the highest* of all the assays and persisted, although of lower order after meal. *The highest "inhibition" of 5-NT-ase* of snake venom *by MS sera* seems to be a *characteristic finding*, as it will be discussed later.

Serum pools (aqu. dilution 1 : 9)	acid phosphatase	alk. phosphatase	5-Nucleotidase
as increase in inorganic Phosphorus µg/ml of system*			
	pH 5	pH 10.9	pH 8.5
Neurol. normal males, 20—65 yrs. of age, pool of 607 individual specimens (frozen)	0.15	0.15	0.32
Neurol. normal females, 20—65 yrs. of age, pool of 650 individual specimens (frozen)	0.15	0.10	0.25
Equal. volume of above pools mixed and assayed	0.20	0.35	0.4
Neurol. normal males, 20—65 yrs. of age, pool of 5 individuals specimens (frozen) *before meal*	0.05	0.55	0.15
after meal	0.25	0.40	0.15
M. S. patients males, 20—65 yrs. of age, pool of 5 specimens (frozen) *before meal*	no activity	0.60	no activity
after meal	0.05	0.75	0.13

Table I. Phosphomonoesterases in normal and MS sera. * For composition of test system and techniques see "methods".

Influence of fasting and food intake

Table III illustrates the behaviour of *individual* fresh MS samples before and after breakfast. A pool of the same specimens was assayed simulatenously. In agreement with the findings presented in Table I, there was no acid ph-ase activity detectable in those tenfold diluted sera, neither before, nor after food intake. There was a moderate activating effect, which varied considerably with the individuals, but decreased after meals in general. The *pools* however behaved somewhat differently from the individual sera, namely their activity *increased* after meals, thus it showed resemblance to the MS pools illustrated in Table II. The alk. ph-ase activity of the same specimens was relatively high. The individual values increased significantly after meals, with exception of a remittent patient (T.I.) on low fat diet[9]. Purified alk. ph-ase was activated by these MS sera, but this effect was drastically reduced after meal. The highest specific influence (>126%) was observed in the pre-meal serum of a chronic remittent patient (F.A.). The highest post-meal value was observed with the serum of a severe catatonic-type of patient (S.M.). Unfortunately there are no fasting values available in this case. The least fluctuation was observed with the patient which was in the best clinical state (T.I. actively working woman, on low fat diet and on Vitamin B_{12} injections). *Pool* of the 5 specimens however behaved *differently* from individual

[9] R. L. Swank, Arch. Neurol. Psychiatry **73**, 630 [1955].

A/assays with normal sera			
Enzyme added 50 μg / test	acid phosphatase as difference betw. calculat.a, found [%]	alk. phosphatase [%[	5-nucleotidase [%]
Neurol. normal males, pool of 607 individual specimens (frozen)	> 65	> 9	> 5
Neurol. normal females, pool of 650 individual specimens (frozen)	> 65	> 2	> 5
equal volume of above pools mixed and assayed	> 48	> 45	
Neurol. normal males, pool of 5 individual specimens (frozen) *before meal*	> 16	> 54	< 7
after meal	> 14	< 11	< 5
B/assays with pathologic sera			
MS patients chronic remittent cases, males (frozen) *before meal*	> 2	> 69	< 9
after meal	> 34	> 14	< 2

Table II. Specific effect of sera on purified or concentrated enzymes.

samples. We could not account for the discrepancy between the post-meal increase of the enzyme activity and the decrease of the activation of purified alk. ph-ase after meals. There seems to be a *correlation with the clinical state*, but obviously this suggestion needs further experimental confirmation.

The 5-nucleotidase activity was relatively high and uniform in pre-meal specimens. Two lower values were observed after food-intake, two were unchanged. There was generally a uniform decrease relative to the calculated activity in serum-snake venom mixtures. This difference seemed to be the *highest* with one of the most *severely ill patients* and was not significant in the pools (Table II).

Effect of handling and storage

Table IV illustrates *various factors* which may influence the specific effect of MS sera. One specimen with considerable mental involvement (encephalopathy?) the activation of acid ph-ase was high (similar to the normal large pools in Table I), the one of alk. ph-ase moderate (lower than the majority of post-meal values in Table III). In contrast to the general pattern (<) 5-NT-ase of snake venoms was activated. *This may suggest the presence or lack of some components* in addition to the MS pathology. The behaviour of haemolytic sera confirms this tentative explanation, exhibiting an inhibition of alk. ph-ase (instead of the costumary activation) and 5-NT-ase was also strikingly activated. *These properties were modified considerably by deep-freezing*, namely marked increase with acid ph-ase and decrease with 5-NT-ase.

The next examples illustrate the effect of shorter and longer contact of the serum with the blood-clot and *storage at 4 °C* on the specific activities of the specimens. The latter serum was reexamined after about 2 months and a *significant increase* in 5-NT-ase inhibition was observed. These findings emphasize the complex nature of this process. The effect of storage is further illustrated with the high degree of inhibition of cobra-venom 5-nucleotidase by sera of chronically paralyzed patients. The decrease of this property in the post-meal specimen of J. B. is not fitting into the pattern observed previously and may be due to the long storage.

The *survey* of the behaviour of 30 specimens under similar circumstances shows 5-NT-ase-inhibition by the majority of MS specimens. Lipaemic samples exhibited only medium range of inhibition. Coordination with the clinical data would be essential for the interpretation of the above findings. For instance relatively fresh case (under 5 years duration) in relatively good physical condition under identical experimental set-up did show only <8%, both before and after meals. The slight degree of activation may be explained on technical basis (experimental error, haemolysis, storage). Similar assays on blood clots [10] confirm the assumption that under similar experimental conditions the corpuscular elements of the blood exert effects entirely different from the sera. These findings may contribute to the explanation of some of the discrepancies observed.

Table V presents examples of experiments carried out on CSF's. *No change* in the activity of enzyme concentrates was observed when incubated together

[10] Unpublished observations.

Patients Name clin. data	acid ph-ase* in serum	spec. effect** of serum on purified ac. ph-ase [%]	alk. ph-ase* in serum [μg/ml]	spec. effect** of serum on purified alk. ph-ase [%]	5-Nucleotid-* ase of serum [μg/ml]	spec. effect** of serum on snake venom 5-NT-ase [%]
F. A. 47 y.*** chronic stationary (in wheelchair) *Before meal*	no activity	> 10	0.25	> 126	0.35	> 1
after meal	,,	> 2	0.50	> 25	0.10	< 2
F. L. 40 y. chronic spastic (bedridden) *before meal*	,,	> 10	0.80	> 50	0.40	< 3
after meal	,,	> 8	1.10	> 3	0.40	> 5
T. I. 45 y. chronic, walking patient on fat-free diet and Vitamine B_{12} *before meal*	,,	> 12	0.80	> 15	0.30	< 6
after meal	,,	> 10	0.80	< 2	0.30	< 10
M. G. A. 28 y. chronic remittent, walking patient *before meal*	,,	> 2	0.40	> 33	—	< 4
after meal	,,	> 2	0.50	> 5	0.13	< 11
S. M. 45 y. chronic severe spastic bedridden *before meal*	—	—	—	—	—	—
after meal	,,	> 4	1.05	> 82	0.40	< 14
Pool of above 5 specimen *before meal*	0.02 μg/ml	> 5	0.70	> 21	0.35	< 7
after meal	0.02 μg/ml	> 8	0.55	> 24	0.30	< 5

Table III. Effect of nutrition on enzyme kinetics in MS unfrozen fresh sera. * Activity as increase in IP μg/ml of system over the values of the controls; for techniques see „methods". ** As % difference between the value expected and found. *** All the specimens were from female patients, sick over 10 yrs., and were stored unfrozen 1 day at 4 °C.

with a *large pool of normal samples.** On the other hand extremely high specific effects were observed with many MS specimens. Typical results are shown in this table, with an *extremely large activation of acid ph-ase*, and strikingly *strong inhibition of 5-NT-ase*. A survey of assays on 17 individual CFS's indicates that these observations are not due to chance, because 16 did display 5-NT-ase inhibition, with extremely high values in about 1/3 of all cases.

* Specimens from neurologically normal persons; they gave negative results with the routine clinical laboratory tests. Courtesy of Mr. KEN SMITH, Division Laboratories, Ont. Department of Health, Toronto, Ont. Canada.

Further research has to account for the variations observed; it seems however, that some biologically active principle is more frequently present or more reactive in the CSF, than in the sera of MS patients.

Discussion

The results published in this report are of preliminary character, because for extraneous reasons the continuation of this work was not possible. There is no statistical interpretation, thus only tentative explanations are offered. This lets the way open for further work, and may stimulate and help

Clinical data	% Difference between values calculated and found activity of		
	acid phosphatase [%]	alk. phosphatase [%]	5 – Nucleotidase [%]
C. A. 50 y. male chronic, spastic walking MS patient (unfrozen specimen)	> 60	> 13	> 9
W. S. 50 y. male chronic remittent MS patient, haemolytic serum *fresh*	> 58	< 8	> 37
frozen	> 73	< 10	> 9
H. R. 32 y. female remittent MS patient in good condition serum 14 days at 4° C over clot (unfrozen)	> 60	> 11	> 19
B. S. 37 y. male chronic remittent MS patient, 72 hrs. at 4° C before assay (unfrozen)	> 72	< 1	< 13 repeated after 60 days storage at 4° C < 50
5-NT-ase assays in specimen stored several months at 4° C			
K. C. 44 y. male chronic MS patient legs paralyzed over 10 y., in wheel-chair sera frozen and thawed, than stored	< 42	J. B. 50 y. male chronic MS patient, legs paralyzed, over 10 y., in wheel-chair (large B_{22} inj) sera frozen and thawed, than stored	*Before after* meal < 70 < 50
MS sera assayed: 30 (for 5-Nucleotidase) Range of inhibition: Very inhibit.: 12 15–73%		Inhibiting: 25 6–73% Moderately: 9 inhibit. (*lipaemic*) 6–15%	
Activating: 5 Range of Activation: 0–36%		Moderately Act. 4 6–15%	Very Act.: 1 36%

Table IV. Factors influencing the specific effect of sera.

clinical workers to explore these or similar questions on hospitalized patients. Variations in the phosphomonoesterase levels of serum pools under many physiological and in some pathological circumstances were described previously [1, 2]. On the basis of those studies [1, 2] the use of large frozen serum pools was recommended. This suggestion seemed to be justified and was governed by the necessity to store the MS sera collected at −25 °C. *This assumption*

Clinical data	% difference between values expected and found		
	acid phosphatase	alk. phosphatase	5 – Nucleotidase (of snake venom
Pool of 141 individual CSF's from neurol. normal persons	∅	∅	∅
B. M. 30 y. male, chronic MS patient, remittent; sample about 2 months at 4° C	> 103	< 42	< 83!
MS. specimens assayed for spec. effect on 5-nucleotidase 17	Inhibiting: 16	Activating: 1	> 36
Range of inhibition in %	< 1–10	< 11–20	< 21–50 < 51–90
Total nb of specimens	5	3	3 5 1

Table V. Specific effect of CSF's of MS patients on enzyme-concentrates.

however was not fully verified during the present studies, because some enzyme activities were of much lower order, then expected [1, 2]. These moderate normal values are due to the tenfold dilution for tests, the interaction of the individual sera, the leveling-off effect of the large number of samples pooled and the *influence of freezing*. Acid and alkaline phases were more sensitive, 5-nucleotidase was more resistant in these respects [5, 6, 11].

Small pools of sera, both from normal persons and from MS patients gave results different from the large pools. This difference may be due to the small number of samples, compared to the large pools [1, 2] but also to the shorter period of storage under more controlled circumstances; namely those 5 specimens derived from *volunteers*, in contrast to the routine material of the large pools.

Furthermore parallel assays of small MS serum pool and its individual samples demonstrated the *discrepancy* between the two experimental materials. The mean value of the results in 5 sera differed not only quantitatively, but also qualitatively, from that of the pool (see also ref. [14]). Thus small and large

[11] Y. IRVING, Trans. N. Y. Acad. Sci. Ser. II **20**, 454 [1958].

pools or individual samples are not directly comparable.

The general absence, or a very *low acid phosphatase* and 5-NT-ase activity in fresh of frozen MS specimens may be of interest in the light of the opinion of various writers, who emphasize the *more specific role* of these enzymes in the nervous tissue [12, 13, 13a].

The specific effect of small and large normal pools on purified or natural enzyme concentrates differed *quantitatively* although they *agreed* in their general tendency. These values are subjected to variations, because they are the product of two activities (enzyme-concentrate and serum). The former may be held relatively constant, the latter varies, for instance with the food-intake or *handling of the samples.* Therefore small differences between calculated and observed activities ($\pm 5\%$) are not considered significant.

There seems to be a real *deviation* from normal in the influence of MS specimens on pure or "natural" enzymes. The response to meals, as regards biocatalytic activities, seems to be also different from normal. Here again the quantitative order was affected more than the qualitative ($>$ or $<$). This was striking with the 5-NT-ase, which showed the most uniform "inhibition" in the MS sera. The results of separately and jointly assayed viper- and serum-5-NT-ase differed about 5 to 14%, but with some stored sera or CSF's 90% difference resulted. *Inhibition* was the general pattern, only exceptionally being replaced by an increase due perhaps to controlable factors. Lipaemia for instance countered this tendency, which fact may be responsible for the *regression of post-meal values.* On the other hand a lipaemic specimen of a normal person (Dg.: cyst of the thyreoid) did show an abnormally high inhibition (19%). This exceptional behaviour may be connected with the basic pathology.

Other factors such as *haemolysis*, or longer contact of serum with the blood-clot caused the MS specimens to lose this property. This effect may depend on other enzymes of the plasma [10, 11] and the snake venom, especially the Phospholipases [7, 14]. In a previous publication we described the decrease of 5-NT-ase activity in the mixture of brain homogenate and snake venom, apparently due to formation of lysophosphatides [7]. Analogous mechanism may operate in MS sera or something similar to that described by MUCH in specimens of neurotics inhibiting cobravenom-haemolysis [15, 15a]. The use of purified 5-NT-ase devoid of phospholipase activity [6] may elucidate this problem [10]. Similarly the low activities in lipaemic MS sera (upon long storage) may be due to endogeneous Lysophosphatid formation [14]. The presence of a haemolytic principle (possibly Lysolecithin) was demonstrated in the majority of MS samples [14] and in some normal specimens [14, 16]. The presence of heparin- or liproprotein like inhibitors [17, 18, 2] in sera, exhibiting alimentary lipaemia, may explain the inhibition of acid ph-ase (for literature see ref. [1]). On the other hand WILMOT and SWANK did not find higher frequency of occurence of lipaemia in MS patients than in normal persons, neither much difference in total lipids or lipid fractions between the two groups [19, 20]. Their findings however do not exclude the possibility of qualitative changes. The curious thing is that some enzymes were enhanced in MS sera (especially after meal) others inhibited and these properties became more accentuated after *storage* at 4 °C. These observations suggest that there may be different phospholipid, lipoprotein and polysaccharide complexes involved in this mechanism [21–25a] which compounds

[12] A. NAIDOO and D. E. BRATT, Enzymologia [Den Haag] **16**, 298 [1958].
[13] G. GOMORI and R. D. CHESSIK, J. Neuropathol. **12**, 387 [1953].
[13a] D. M. HOLLINGER, R. J. ROSSITER and H. UPMALIS, Biochem. J. **52**, 652 [1952].
[14] E. KOVÁCS, Z. Naturforschg. **14 b**, 550 [1959].
[15] H. MUCH, Die Pathol.-Biologie, Verlag Kurt Kebritzsch, Leipzig 1912, S. 240.
[15a] P. T. MÜLLER, Vorlesungen über Infektion und Immunität, IV. Aufl., Gustav Fischer, Jena 1912, S. 434.
[16] G. B. PHILLIPS, Proc. nat. Acad. Sci. USA **43**, (No. 7), 566 [1957].
[17] J. S. ROTH, Arch. Biochem. Biophysics **44**, 265 [1953].
[18] J. BORNSTEIN, Biochim. biophysica Acta [Amsterdam] **20**, 522 [1956].
[19] V. C. WILMOT and R. L. SWANK, Amer. J. med. Sci. **223**, 25 [1952].
[20] R. L. SWANK, Science [Washington] **120**, 427 [1957].
[20a] R. L. SWANK, Amer. J. Physiol. **164**, 798 [1951].
[21] E. A. KABAT, D. A. FRIEDMAN, J. P. MURRAY and V. KNAUP, Amer. J. med. Sci. **219**, 55 [1950].
[22] R. L. SWANK, Arch. Neurol. Psychiatry **69**, 281 [1953].
[23] R. B. AIRD, J. W. GOFMAN, H. B. JONES, M. B. CAMPBELL and B. GAROUTTE, Neurology [Minneapolis] **3**, 22 [1953].
[24] A. SAIFER, A. M. RABINER, J. ORESKES and B. W. VOLK, Amer. J. med. Sci. **225**, 287 [1953].
[25] E. ROBOZ, W. C. HESS and F. M. FORSTER, Neurology [Minneapolis] **3**, 410 [1953].
[25a] R. R. APOSTOL, E. ROBOZ, W. C. HESS and F. M. FORSTER, Neurology [Minneapolis] **6**, 859 [1956].

may undergo changes during storage especially at low temperatures.

There is seemingly a connection with the clinical state or stage as regards enzyme activities, respectively the presence of substances of specific kinetical effects. Other findings suggest that the latter may be perpetually present, but *hold in check* serologically. One could assume, that this activators or inhibitors may be part of an *immunologic defence mechanism* generated or provocated by the basic pathology. An other alternative would be that the whole process might be caused by a *cyto- or enzyme toxic principle*, eventually similar to lysophosphatides, as the consequence of faulty lipid-metabolism [14]. Obviously more data are needed to answer these questions. Preliminary assays with brain homogenates + MS sera did reveal some differences from normal [10]. Perhaps the use of purified normal human enzymes (serum and nerve tissue) would be a suitable experimental tool for the elucidation of this problem.

The role of liver may be an other important, although obscure factor in the etiology resp. symptomatology of MS [26, 27, 27a]. It remains to be decided if a connection exists between the postmeal enzyme-changes observed and a disturbance of hepatic function. Spectrophotometric studies on MS sera (not yet concluded) did not show essential differences from the normal [10].

The high kinetical effect of the CSF's of MS patients is a very suggestive finding. We do not know if there is an *elective* accumulation of these biochemically active principles, or the higher activity is due to the different composition of the CSF, relative to the serum. Further work may allow perhaps to distinguish between the *primary or secondary* character of the processes described. Careful correlation of the clinical and the biochemical events may allow a progress in this direction, however the exploration of the behaviour of fresh individual normal specimens (both sera and CSF) under various experimental conditions is the sinequanon of further successful work.

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Conclusions

On ground of these preliminary studies it seems that phosphomonoesterase, especially the acid phosphatase activities in specimens of MS patients *may differ* from those of normal individuals. There is also a difference in the effect of MS samples on the *kinetics* of purified acid and alkaline phosphatase and on snake venom 5-nucleotidase, as compared with those of normal controls. It was not decided, whether single or multiple biochemical factors are responsible for the reported observations; they seem to be present in variable amounts in the MS sera and CFS's. This variability may depend on clinical condition, stage, duration and form of the disease as well as food intake and handling of the specimens (especially cold storage). An additional great number of carefully selected unfrozen individual samples of normal persons and MS patients has to be investigated under standarized experimental conditions to asses the statistical significance of the findings described.

[26] R. M. Brickner, Arch. Neurol. Psychiatry **23**, 715 [1930].
[27] L. A. Crandall and A. Weil, Arch. Neurol. Psychiatry **29**, 1066 [1933].
[27a] L. A. Crandal and I. S. Cherry, Arch. Neurol. Psychiatry **27**, 367 [1932].