SERUM GLYCOPROTEINS IN RHEUMATIC CHILDREN

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The proper treatment of children affected with rheumatism and the guidance of curative and prophylactical measures in the postrheumatic attack period require early diagnosis of the oncoming rheumatic affection, as well as exact paraclinical and clinical indices ensuring full control of the evolution of the disease. The modern treatment of rheumatism and the extensive use of corticosteroid hormones permit a comparatively prompt effect on subjective complaints, objective findings and on certain paraclinical indices such as erythrocyte sedimentation rate (ESR), blood picture, most of the protein profile indices etc. Obviously during the brief period of clinical improvement, it is difficult to assert the development of adequate changes of the pathologically damaged tissues. The data of the morphological studies unambiguously emphasize the persistance of the rheumatic evolution which exceeds considerably the universally accepted clinical period. This calls for the search and introduction in practice of a number of new biochemical indices, endowed with greater possibilities than the laboratory tests hitherto employed for assessment of rheumatic activity both in the acute phase and in the later phases of the rheumatic process development. In recent years the attention of many authors was focused on the study of serum glycoproteins.

The glycoproteins (GP) represent complex compounds of polysaccharide structures with a protein. Their physico-chemical properties are comparatively well studied (12, 16, 18) but the metabolism of GP is by no means clarified (22). It is accepted that the polysaccharide structures are formed mainly within the connective tissue and more particulary in the fibroblasts with the final structural build up of the GP taking place in a number of visceral organs — mainly in the liver (4, 8, 13, 20, 23). Other organs are likewise worth of note, such as the cerebral and renal tissue (10), the vascular wall, intestines, salivary glands etc. In infectious-allergic affections the tissue metabolism is disturbed by depolymerization of the glycoproteid complexes (19, 21). The increased serum values of the glycoproteid components such as orrhosomucoid haptoglobins, sialic acid, fucose, hexosamines etc., are explained, on the one hand, by this depolymerization and, on the other hand, by the intensified synthesis. The setting into motion of the mechanisms of synthesis is maintained in the different stages of the inflammatory process by the respective predominance of alterative and proliferative changes which not infrequently, remain beyond the control of clinical observation (5). The latter fact determines the diagnostical value and clinical importance of the serum GP investigation in a number of diseases of the connective tissue of the cardio-vascular system and the gastro-intestinal tract, liver, kidneys and lungs.

Studies on serum GP in adult rheumatic patients in Bulgaria have been performed heretofore by *Sv. Razboynikov, A. Karakashov and associates.*

In the present work we set out to investigate consecutively the possibilities of serum GP in the diagnostics of rheumatism in childhood and their clinical importance as rheumatic indices, both in the active phase of the disease and in the later phases of development in the post-attack in the free-of-attack periods of rheumatic disease.

Material and Method

In the course of two years a series of 181 childern aged from 3—14 years were investigated; of the total number 132 children were with rheumatic affections, 16 — with suspected rheumatism, 18 — with chronic tonsillogenic intoxication and 15 — with congenital heart defects. In addition, during same period, 110 healthy children of comparable age underwent investigation for control purposes.

Of the rheumatic cases 89 children were with a first attack, 32 — with second attacks and 11 — with more than two attacks. 83 children were in the active phase of the disease, 27 — in the post-attack period and 22 — in the period free of attacks (Table 1). 113 children were with clinical,

Table 1

Investigation of rheumatic childern distributed according to phases and clinical forms of the affection

Phases of the disease	Investigated children	Evidence for cardiac affection			No cardiac affections	
		Cardiac-articular form	Cardiac form	Cardiac-nervous form	Articular form	Nervous form
Active phase	83	66	5	2	6	4
Post-attack period	27	19	2	1	3	2
Period free of attacks	22	15	2	1	2	2
Total	132	100	9	4	11	8

paraclinical and electrocardiographic data for heart affection, distributed in the following clinical forms: heart articular form — 100, cardiac form — 9, cardio-nervous form — 4. The remainder (19 children) were without data about heart affection: articular form — 11, nervous form (chorea minor) — eight. Seventy three children had data for active rheumocarditis with established heart defect — 29 with cardio-vascular insufficiency II and III degree — 14. With proved focal infection from tonsillogenic and dental origin 38 children. During hospitalization the children underwent treatment with penicillin, benzacyllin, amidophen, dehydrocortisone, vit. C, vit. B_1 and in case of need with cardiac glucosides, after which they were transferred to the specialized rehabilitation rheumatic hospital in the village of Suvorovo for continuation of the treatment. Here prophylaxis and rehabilitation of the rheumatic children was carried out in the post-attack and free-of-attack periods.

Of all the glycoproteid blood serum constituents we studied: 1) orrhosomucoid (α_1—acid GP) — according to the method of *Dechosal* in mg % as tyrosine; 2) haptoglobins (hp) — according to the method of *Turchenko and Tukachinskii*. The investigation of orhosomucoid (OM) and Hp reflect up to a great extent the changes in the protein components of the serum GP. As indices demonstrating the variations in the polysaccharide components of serum GP we studied: 1) sialic acid (SA) according to the thiobarbituric method of Aminoff; 2) hexosamines (Ha) — according to the method of Elson, Morgan, Rimington and 3) fucose (Fc) — according to the method of Dische.

The normal values of serum GP in the control group of healthy children are illustrated in Table 2. For SA and Hp the results obtained in healthy

Table 2

Normal values of serum glycoproteins (in mg %) according to various authors and personal data

Serum glycoproteins	Authors	Personal data
Sialic acid	66,7±6,1 (L. Warren)	49,9±14,8
Haptoglobins	105±45 (E. Boyadjiev)	125,5±60,4
Hexosamines	70—100 (Elson — Morgan / Rimington)	47,9—111,0
Glycoproteins	3,38±0,27 (R. Winzler)	2,0—4,7
Fucose	8,31±2,24 (Z. Dische)	2,7—10,3

children had normal distribution and the normal values are submitted through mean value ± 2. For OM, Ha and Fc the distribution was lognormal and the range of normal values is computed by means of logarithmic transformation (3).

The statistical elaboration of the results obtained during the investigation of serum GP was made by the following methods: arithmetical mean value analysis, standard deviation evaluation and comparison of mean values and percentages (7).

Results and Discussion

In Table 3 the mean values with standard deviation of serum GP are given as compared to those of ESR in the first ten days of the rheumatic process in children suspected for rheumatism, in cases with chronic tonsillogenic intoxication, congenital heart defects and in a control group of healthy children. It is evident from the analysis of the results that together with ESR, the mean values of serum GP are highest in rheumatic children. Particulary high was the serum level of GP in children with active rheumocarditis (twice as high as that of the control group of healthy children). In children free of heart affections (articular and nervous form of rheumatism) the mean serum GP values were less elevated. Out of 16 investigated children with doubtful rheumatism, only 3 produced high values of the serum

GP and the subsequent clinical follow-up and paraclinical investigations proved that a rheumatic process existed. The remaining 13 children proved to be with various non-rheumatic diseases (catarrhs of the upper respiratory ways, accompanied by arthralgias and vague pains in the limbs, tachy-

Table 3

Mean values of erythrocyte sedimentation rate and serum glycoproteins (in mg %) in the first 10 days of the rheumatic process, rheumatism suspects, chronic tonsillogenic intoxication, congenital cardiac disease and control group of healthy childern

Serum glycoproteins	Rheumatism		Suspectful for rheumatism	Chronic tonsillogenic intoxication	Congen. heart diseases	Control group of healthy children
	with active rheumocarditis	without heart affections				
ESR mm/1 h after Panchenko	58,6 ± 12,1	29,9 ± 10,2	20,3 ± 7,4	15,9 ± 8,1	11,7 ± 6,2	7,6 ± 4,7
Orrhosomucoid	5,9 ± 1,1	4,2 ± 0,8	3,9 ± 0,5	3,1 ± 0,8	3,4 ± 0,7	3,2 ± 0,7
Haptoglobins	227,9 ± 31,6	154,4 ± 22,1	118,3 ± 21,2	122,8 ± 25,7	114,7 ± 18,6	125,5 ± 30,2
Sialic acid	79,9 ± 9,4	52,7 ± 9,2	49,1 ± 5,3	50,1 ± 6,2	44,1 ± 7,5	49,9 ± 7,4
Hexosamines	138,1 ± 16,6	122,1 ± 8,4	101,2 ± 14,8	107,1 ± 16,4	78,3 ± 12,6	74 ± 16,4
Fucose	11,7 ± 1,8	9,6 ± 1,8	7,5 ± 1,4	7,1 ± 1,5	6,3 ± 1,2	5,5 ± 1,6

cardia, accelerated ESR etc. The investigation of children with chronic tonsillogenic intoxication disclosed insignificant variations from the normal values merely in the carbohydrate components of serum GP (SA, Ha, Fc). In the children with congenital heart defects the values of the serum GP proved to be within normal limits. Hence the inference is made that determination of serum GP may be employed as a valuable diagnostical criterion in the complex investigation of rheumatic children in the early, acute phase of the disease.

In Fig. 1, the dynamics of the mean values of ESR and serum GP are graphically given by days and months in the course of the rheumatic process. The highest values of serum GP are observed during the first ten days, except for Fc where the peak of the dynamic curve is recorded about the 20th day from the commencement of the disease. The serum level of GP follows the dynamics of ESR. Whilst in ESR the mean values after the 30th day fall beneath 20 mm after Panchenko, the values of serum GP are maintained at a higher level. After the 40—50th day, a slight increase is noted of the serum GP level, which coincides with the discontinuation of the corticosteroid therapy. A similar slight elevation of values of serum GP is marked also after the fifth month which, in all likelihood, is related to the cessation of the active treatment of rheumatism and the passage to prophylactic measures. These changes are absent in the ESR dynamic or are but slightly noticeable.

In Figure 2 through a step-like diagram, the percentages of the positive results of blood serum glycoproteid components are presented in dynamics, namely: α_1 — acid GP (orrhosomucoid) and haptoglobins (Hp) compared to the

positive ESR values. Within 10 days of the onset of the disease, 90% of the rheumatic children disclosed high values of Hp and 79% — high values of orrhosomucoid (OM). During the following days, the percentages of the positive results began to decrease together with those of the ESR with the most rapid decrease being noted in the Hp values. After 30th day the positive results of Hp were rapidly reduced below 16%, followed by those of ESR — 32% and OM — 50 per cent. This dissociation between the values of Hp ESR and OM is also maintained in the later stages of the development of the rheumatic process. The phenomenon is particulary clearcut in Figure 3 from which it becomes evident that the normalization of Hp precedes that of ESR whilst the values of OM are maintained comparatively high. Along with that, it is our impression that the dynamic curves of the Hp and OM run more ondulated course as compared to that of ESR. After the 40th day a stabilization of the OM serum level is observed and a tendency towards slight increase of Hp as well which coincides with the discontinuation of corticosteroid therapy. Within 50 days the positive results rapidly regain their normal values. The application of steroid hormones and the subsequent inhibition of the proliferative changes in the tissue lead to considerable restriction of ESR and Hp variations. This may be considered as a reliable indication of the successful hormonal treatment. Not infrequently the hormonal treatment discontinuation is being manifested by the so-called reverse phenomenon rechute which may remain merely at biological level and will account for the arrest or slight increase of the serum GP values without pronounced clinical manifestations and without changes in the ESR dynamics. The rapid transitory nature of these changes lends support to the opinion that a particular case of short-lasting relapse is concerned and not a recurrence. The tracing up of the Hp and OM dynamics produces valuable information about the manifestation of the reverse phenomenon.

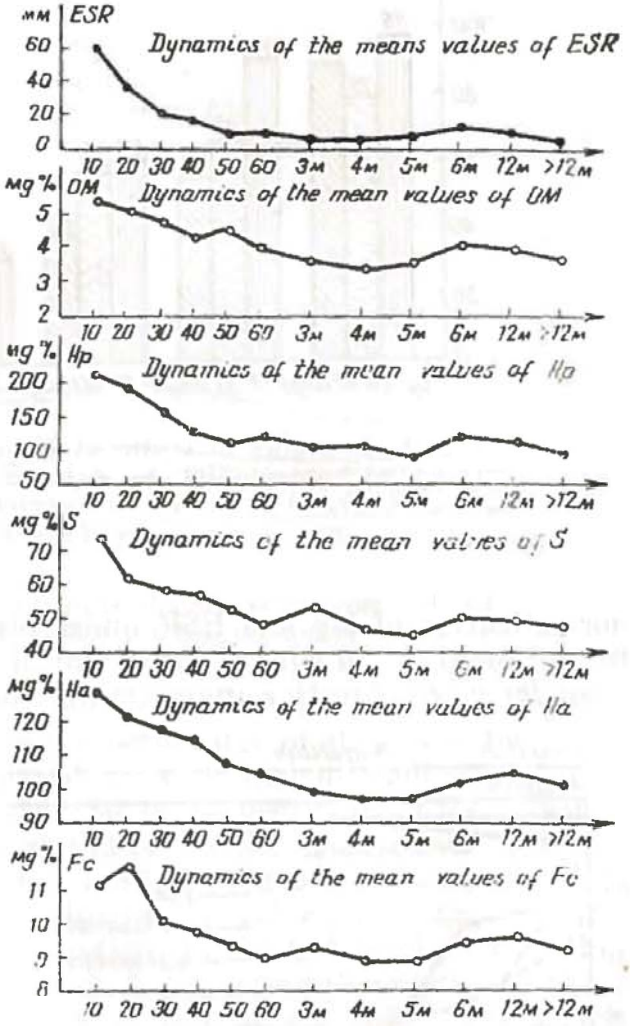

Fig. 1. Dynamics of the mean values of erythrocyte sedimentation rate and serum glycoproteins according to days and monts in the course of a rheumatic process.

The analysis of the positive results of Hp and OM in the first 60 days from the commencement of the rheumatic process shows that their determination may be employed as a reliable index for the rheumatic process activity in the early acute phase of the disease. The comparatively prompt

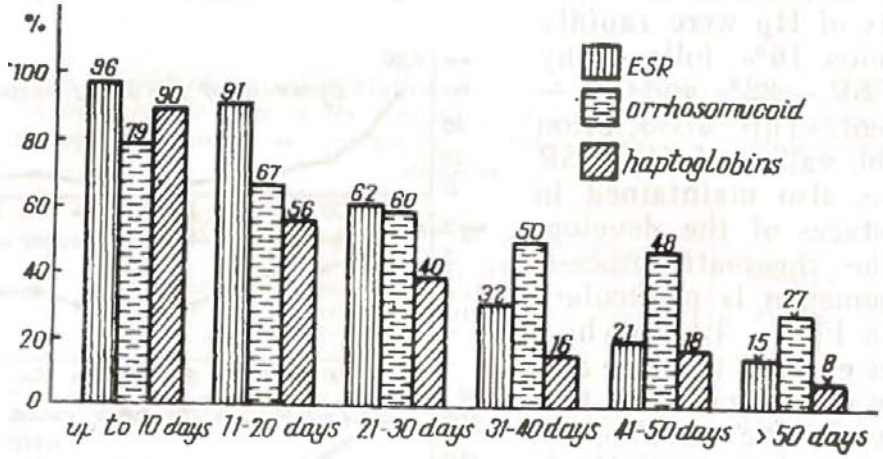

Fig. 2. Dynamics of erythrocyte sedimentation rate, orrhosomucoid and haptoglobins by days in the course of the rheumatic process. The positive results in percentuals are submitted on the ordinate.

normalization of Hp and ESR under the effect of corticosteroid therapy is not followed by an equally rapid fall of the OM serum level. Therefore OM provides more objective indication of the reverse development of the rheumatic process in the course of hormonal treatment. In slighter and less complicated forms of rheumatism, OM values are normalised about the 60th day after the beginning of the attack. In instances of heavier and protracted course these values are retained at a higher level for a long time and reflect to certain extent the fluctuations, characteristic for the evolution of the process. Unlike ESR the serum OM level is but slightly influenced by the cardio-vascular insufficiency and represents a reliable rheumatic activity index in cases of cardio-vascular decompensation.

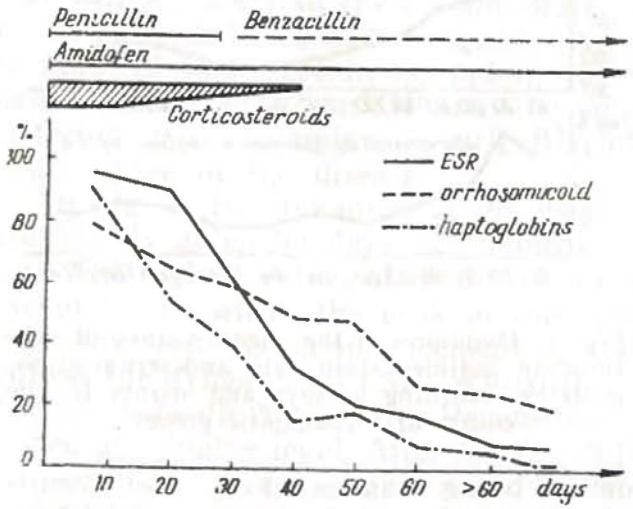

Fig. 3. Dynamics of the positive results of ERS, orrhosomucoid and haptoglobins by days since the onset of the disease.

The dynamics of the positive results for Hp and OM are illustrated monthly in the course of the rheumatic process in Fig. 4. Whereas after the first month the values of ESR and Hp show a rapid decrease those of OM are retained at a higher level, making it possible to trace up the course of

the rheumatic process and its involution over a longer period of time. After the first month the raised serum level of Hp shows a rapid decrease to 10% in the second month and below 2% in the children investigated during the third month; thereafter up to the sixth month and after the sixth

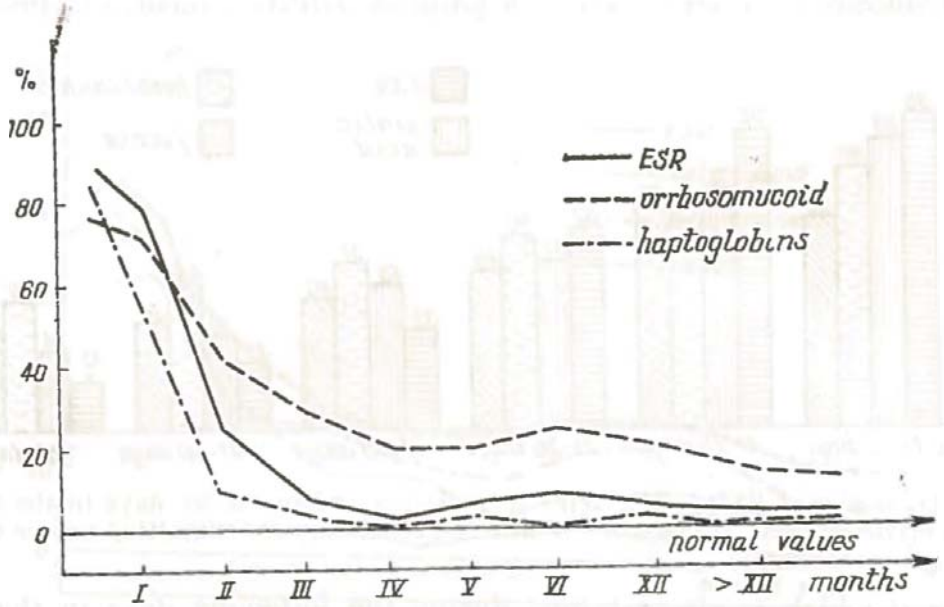

Fig. 4. Dynamics of the positive results of ESR, orrhosomucoid and haptoglobins by months in the course of the rheumatic process.

month, the Hp level fluctuates within normal limits. The children investigated in the post-attack period and in the period free of attacks of the rheumatic disease disclosed normal Hp values with insignificant variations. The parallel dynamics curve of Hp and ESR in the beginning of the rheumatic attack outlines their importance as indices of the acute phase. The dynamic changes in the OM level show a tendency for a comparatively continuous maintainance of high values also in the subsequent stages of the rheumatic disease. The considerable variations in the OM levels during this period may be assumed as a serious signal for persisting rheumatic activity. The analysis of the positive results in the post-attack and free-of-attack periods of rheumatism demonstrates that the OM values are being influenced also by some intercurrent infections (catarrhs of the airways, viral inrections, tonsillitis, sinuitis etc.) These additional infections in children with rheumatism lead to a rapid transitory increase of the serum level of OM, which after adequate treatment regains quickly its normal values. In instances of rather persisting elevated OM level in the serum, substantially exceeding the norm, the possibility should always be considered of recurrence of the rheumatic process or else of impending new attack of the disease regardless of the absence of clinical evidence.

In Figure 5 the step-like diagram shows in dynamics the percentages of the positive results of the serum GP carbohydrate components, namely sialic acid (SA), hexosamines (Ha) and fucose (Fc) as compared to the positive ESR values. During the first 10 days from the commencement of the disease, 88% of the rheumatic children displayed high SA values, 80% —

high values of Ha and 65% — high values of Fc. During the subsequent days, the percentages of the positive results began to decrease together with those of ESR. After the 30th day the positive results of ESR were sharply lowered whilst those of SA, Ha and Fc were maintained at a comparatively

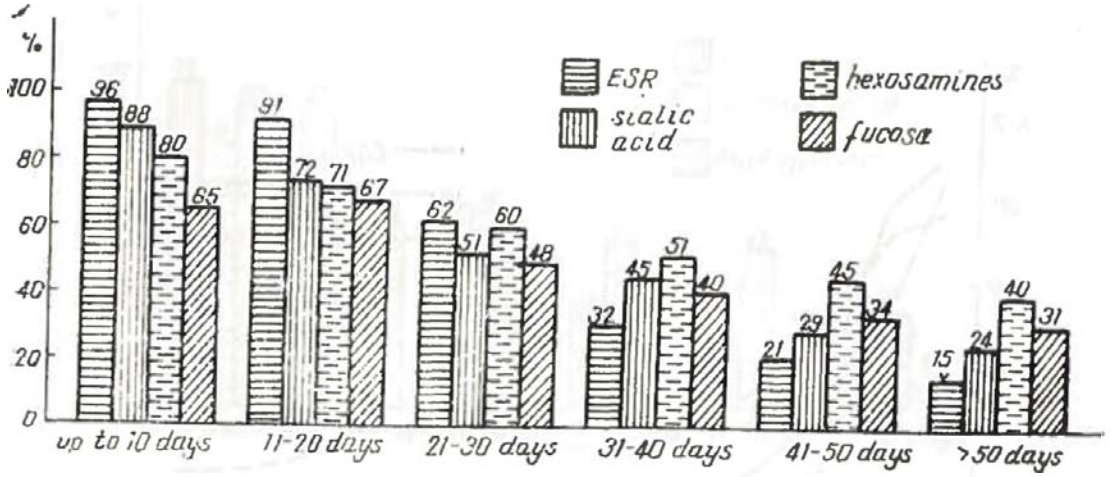

Fig. 5. Dynamics of ESR, sialic acid, hexosamines and fucose by days in the course of the rheumatic process. The positive results in percentuals are submitted on the ordinate.

high level which is also retained during the following days in the course of the rheumatic process. This is evident also in Figure 6 presenting the dynamics curves of these indices. After the 20th day the ESR values began to fall rapidly and about the 30th day the dynamical curve of ESR intersects those of the remaining indices with a tendency towards prompt normalization, preceding that of SA, Ha and Fc.

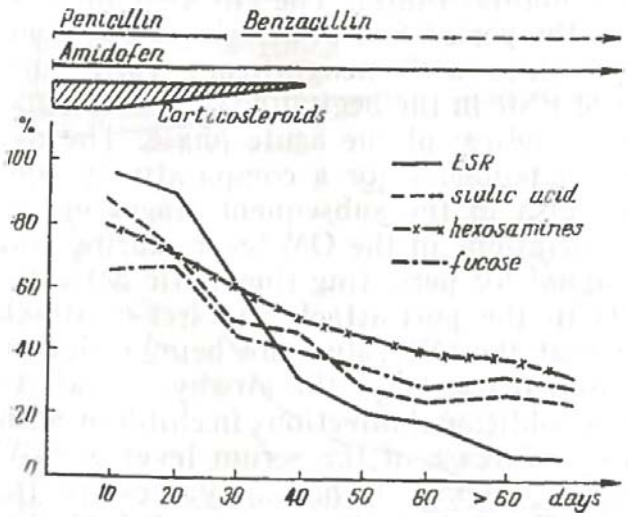

Fig. 6. Dynamics of the positive results of ESR, sialic acid, hexosamines and fucose by days since the onset of the disease.

The analysis of the positive results of SA, Ha, Fc in the first 30 dyas from the commencement of the rheumatic process shows that they may be used as indices of the acute phase and that they follow closely the dynamics of ESR with positive values percentages being the highest with SA, next ranking Ha and Fc. These indices assume a greater clinical importance in the latter stages of the rheumatic disease when their values substantially exceed those of ESR and make possible the follow up of the evolution of the disease for a longer time. Similarly to alpha-1 acid GP (orrhosomucoid) the dynamics of these indices is determined up to a great extent by the nature and severity of the process and is less influenced by the antirheumatic treatment and cardio-vascular insifficiency. The

substantial deviations in the values of SA, Ha and particulary Fc indicate heavy impairment of the GP metabolism and afford valuable information about the activity of the rheumatic process in the latter stages of its development. In rheumocarditis, running a severe course with chronic insuffi-

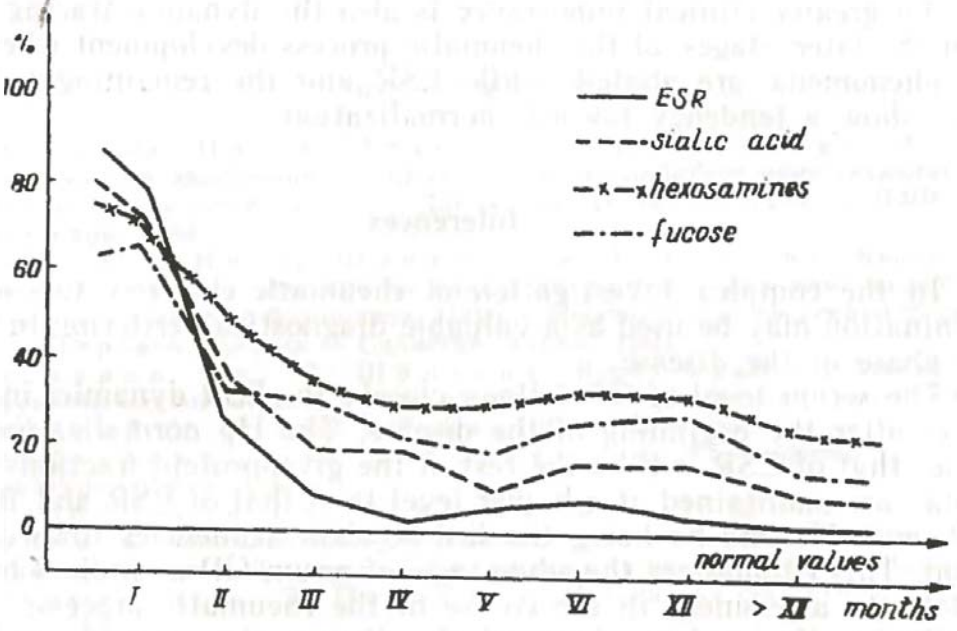

Fig. 7. Dynamics of the positive results of ESR, sialic acid, hexosamines and fucose by months in the course of the rheumatic process.

ciency of circulation and early mitral vitium, the normalization of these indices takes place after a prolonged period of time.

Figure 7 shows the dynamics of the positive results of SA, Ha and Fc by months in the course of the rheumatic process. Whereas after the first month the ESR values are quickly lowered, those of the remaining indices are maintained at a higher level for a long time which is a further proof of the advantages of these indices making possible a complete control of the rheumatic disease evolution in the late stages of its development when there is a considerable lack of symptoms in the clinical and paraclinical pictures. After ESR and SA, Fc and Ha are among the last to show a tendency towards normalization. The persisting of moderately abnormal indices during the post-attack period reflects the rheumatic evolution which, in terms of duration by far exceeds the period of pronounced clinical manifestation. The analysis of the positive results in the post-attack period and in the period free-of-attacks of the rheumatism shows that as in the case of orrhosomucoid, SA values are being influenced by some additional infections in the children affected with rheumatism (catarrhs of the respiratory ways, viral infections, tonsillitis, sinuitis etc.), accounting for a transitory (quickly subsiding) increase of the serum level of SA. The continuously maintained higher level of SA associated to variations in Ha and Fc values is considered as warning signal for recurrence of the rheumatic process or as the beginning of a new attack of the disease.

In conclusion it might be stated that the complex investigation of serum GP at the beginning of the rheumatic attack with a clearly outlined

clinical and paraclinical picture has a more or less corroborative character. The rheumatic attacks running a heavier course are distinguished by the considerable variations in the serum level of Gp and by their greatly delayed tendency towards normalization in spite of the applied hormonal treatment. Of greater clinical importance is also the dynamic tracing of serum GP in the later stages of the rheumatic process development when the clinical phenomena are abated while ESR and the remaining paraclinical indices show a tendency towards normalization.

Inferences

1. In the complex investigation of rheumatic children, the serum GP determination may be used as a valuable diagnostical criterion in the early acute phase of the disease.
2. The serum level of GP follows closely the ESR dynamics in the first 30 days after the beginning of the disease. The Hp normalization usually precedes that of ESR, whilst the rest of the glycoproteid fractions after the 30th day are maintained at a higher level than that of ESR and for a long period, with Ha and Fc being the last to show tendencies towards normalization. This emphasizes the advantages of serum GP as indices for rheumatic activity assessment in the course of the rheumatic process.
3. During the various stages of the disease, the separate glycoproteid fractions have different relative values. In the most acute phase along with ESR, utmost importance is attributed also to the changes in the serum level of Hp, OM and SA. In the latter stages of development with the passing from predominantly exudative to proliferative tissue changes, along with OM and SA, the determination of Ha and Fc assumes special importance.
4. The dynamic follow-up of the GP serum level and more particulary that of OM and Hp gives valuable information concerning the effect of the treatment carried out and the appearance of the reverse, phenomenon following discontinuation of corticosteroid therapy, drawing attention to the appearance of a new deterioration or intensification of the rheumatic disease.
5. Unlike ESR, the serum GP reflects more truly the reverse development of the rheumatic process during the hormonal treatment also in cases with cardio-vascular decompensation. In a more severe and protracted course of the disease, the serum GP values are maintained rather high for a long time, revealing wave-like fluctuations and reflecting more fully the multiformity of the evolution of the rheumatic fever.
6. The approach in cases with delayed normalization of the serum GP imposes careful consideration, prolonged complex treatment and systematic prophylaxis of rheumatic children.
7. The dynamic tracing of serum GP provides valuable information concerning the activity of the process not only in the active phase of the disease, but also in the post-attack and free-of-attack periods when the clinical and paraclimical pictures lack symptoms, thereby focusing our attention towards the appearance of eventual recurrence or secondary attacks of the rheumatic fever.

8. Taking into consideration all the paraclinical possibilities of serum GP, their investigation and dynamic follow-up should be integrated in the complex of laboratory methods for early diagnosis and a complete control of the rheumatic fever evolution.

REFERENCES

1. Даскалов, Ив., Ф. Атанасова, П. Шипкова. Върху серумното ниво и диагностична стойност на хаптоглобините през активната фаза на ревматизма в детска възраст. Доклад на III научна сесия на ВМИ — Варна, октомври, 1968.
2. Даскалов, Ив., П. Шипкова, Ф. Атанасова. Върху серумното ниво и клинично значение на сиаловата киселина при комплексното изследване на ревматично болни деца. Научно-практическа конференция по ревматизма при Окръжна болница — Силистра, април, 1969.
3. Даскалов, Ив., П. Шипкова. Върху нормалните стойности на серумните гликопротеини в детска възраст. Непубликувани данни, 1968.
4. Ларский, Э. Г. *Вопр. мед. хим.*, 1961, VII, 5, 505.
5. Разбойников, Св., А. Каракашов. Гликопротеини, Мед. и физкултура, София, 1967.
6. Разбойников, Св., А. Каракашов, Т. Шипков, Й. Шиварова. Сравнително изследване клиничната стойност на някои съставки на гликопротеините, Доклад на III симпозиум по ревматология на Варшава, 1966.
7. Сепетлиев, Д., Т. Паскалев. Медицинска статистика. Мед. и физк., С., 1968.
8. Тустановский, А., Ф. Боранова. *Вопр. ревматизма*, 1964, 3, 20.
9. Турченко, И. И., С. Е. Тукачинский. *Лаб. дело*, 1966, 4, 195.
10. Цветкова, И. В. *Вопр. мед. хим.*, 1961, VII, 1, 3.
11. Aminoff, D. *Virology*, 1959, 7, 355.
12. Barker, S. A. et al. *Nature*, 1963, 197, 231.
13. Borel, I. P., N. Muray, J. Moretti, M. F. Jayle. C. R. Acad. Sci., 1964, 259, 3857.
14. Dechosal, R. Lecog. Manuel d'analyses medicales et le Biologie clinique, Paris, 1962, 710.
15. Dische, Z., L. B. Shettles. *J. Biol. Chem.*, 1948, 175, 595.
16. Dische, Z. *Exp. annuels de biochimie medicale*, 1963, 24, 49.
17. Elson, Morgan, Rimington, R. S. Winzler, B. D. Glick. Methods of Biochem. Analysis, London, 1957, II, 289.
18. Gottschalk, A. *Perspectives Biol. Med.*, 1962, 5, 622.
19. Jayle, M. F. Les haptoglobines, Paris, Masson et Cie, 1962.
20. Musil, J. *Clin. Chim. Acta*, 1961, 6, 508.
21. Pirani, C., H. R. Catchpole. *AMA Arch. Pathol.*, 1951, 51, 597.
22. Robert, L., B. Robert. *Exp. annuels de biochimie medicale*, 1963, 24, 271.
23. Sonnet, J. Les glycoproteines seriques a l'etat normal et patologique, Bruxelles, 1956.

О СЫВОРОТОЧНЫХ ГЛЮКОПРОТЕИНАХ ПРИ РЕВМАТИЗМЕ У ДЕТЕЙ

Ив. Даскалов, Ф. Атанасова, П. Шипкова

РЕЗЮМЕ

Авторы исследовали сывороточные глюкопротеины (оросомуконд, гаптоглобины, сиаловая кислота, гексозамины и фруктоза) у 181 ребенка в возрасте от 3 до 14 лет, из которых 132 ребенка с ревматическим заболеванием, 16 — сомнительных на ревматизм, 18 — с хронический тонзилогенной интоксикацией и 15 — врожденными сердечными пороками. В течение того-же периода исследовано и 110 здоровых детей соответствующего возраста — как контрольная группа. Исследования прослежены динамически, как в течение активной фазы, так и во время послеприступного и внеприступного периодов ревматизма. Полученные результаты обработаны статистически и иллюстрованы 3 таблицами и 7 рисунками.

В заключении авторы подчеркнивают диагностическое значение повышенных сывороточных глюкопротеинов и их клиническое значение как ценных показателей ревматической активности не только во время острой фазы, а и на более поздних этапах развития ревматического процеса, когда клиника и параклиника очень бедны симптомами. Прослеживание в динамике сывороточного уровня глюкопротеинов отражает более реально обработное развитие ревматического процеса во время лечения гормонами, в случаях с сердечно-сосудистой недостаточностью, при более тяжелом и затяжелом течении заболевания и дает возможность производить полный контроль над эволюцией ревматической болезни.