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Cytostatic Treatment of Nephrotic Syndrome in Children

By

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Ten patients with idiopathic nephrotic syndrome, three patients with nephrotic syndrome due to chronic nephritis, and two patients with nephrotic syndrome due to purpuric nephritis have been subjected to combined cyclophosphamide—prednisolone treatment. It was successful in the 10 cases of idiopathic nephrotic syndrome, but produced no effect in 6 weeks on the condition of the other 5 patients. The histological picture of the kidneys, the results of immune-histological studies data regarding the selectivity of proteinuria, and the prognostic value of the latter are discussed. Haematological and immunological changes, observed in the course of treatment, are described. The increased incidence of chromosomal aberrations registered in connection with the treatment, pointed to the risks of cyclophosphamide therapy.

As a result of immunological studies of the nature of glomerular changes, immuno-suppressive therapy has been adopted for the management of glomerulonephritis. Kelley and Panos [17] were the first to prescribe mustard nitrogen for the treatment of children suffering from the nephrotic syndrome (to be termed NS in the following). In 1963, Goldbeck [5] described the therapeutic usefulness of alkylating agents. Since then, the two most frequently employed such agents, namely cyclophosphamide [7, 9, 28, 43] and chlorambucil [2, 4, 20, 21], have formed the subject of numerous reports. These cytostatic compounds are administered in combination with prednisolone. Several authors obtained good results from antimetabolites such as 6-mercaptopurine and azathioprine which are usually combined with alkylating agents [13, 29, 31, 47]. The combination of azathioprine with prednisolone alone produced no therapeutic effect on the NS of children [1].

Combined treatment with cytostatics (alkylating agents or antimetabolites + prednisolone) are referred to as immuno-suppressive therapy by certain authors [34, 40, 42], although it is doubtful to what extent their favourable effect is due to their immuno-suppressive action.

Steroid-resistant NS patients have been treated by us with cytostatic drugs and the present paper presents the results of the first 15 cases. The treatment is burdensome, and we

TABLE I

Data of patients suffering from nephrotic syndrome

No.	Age (years) sex	Diagnosis	Duration of disease	Maximum of protein-uria g/24 hrs/ 1.73 m²	Minimum total serum protein g per 100 ml	Minimum serum albumin g per 100 ml	Maximum serum cholesterol mg per 100 ml	Endogenous creatine clearance and concentrating power	Haematuria Addis count million/24 hrs	Steroid sensitivity
1	17 ♂	I.N.S.	14 years	19.1	2.2	0.3	870	normal	0.4	A
2	15 ♂	I.N.S.	9 years	17.8	3.2	0.6	680	normal	1.1	B
3	6 ♀	I.N.S.	4 years	16.8	3.3	0.4	560	normal	0.5	C
4	15 ♂	I.N.S.	3 years	16.1	2.9	0.2	490	normal	—	B
5	14 ♀	I.N.S.	3 years	14.0	3.6	1.0	760	normal	2.5	B—C
6	14 ♂	I.N.S.	? ?	5.8	4.7	1.8	500	normal	0.5	D
7	14 ♀	I.N.S.	8 months	11.3	4.3	1.6	760	normal	1.2	C—D
8	14 ♂	I.N.S.	10 years	17.0	2.5	0.5	540	normal	0.3	A
9	16 ♂	I.N.S.	13 years	18.5	2.7	0.4	630	normal	0.3	A—B
10	15 ♂	I.N.S.	18 months	9.9	3.6	1.2	660	normal	1.0	B
11	15 ♂	Chr.N.	2 years	14.1	5.0	2.3	210	constricted	>10.0	D
12	15 ♂	Chr.N.	8 years	5.9	4.9	2.0	300	normal	>10.0	D
13	15 ♀	Chr.N.	7 years	16.7	3.3	1.3	560	constricted	>10.0	D
14	9 ♂	Purp.N.	5 months	15.8	2.2	0.7	480	normal	>10.0	D
15	8 ö	Purp.N.	5 months	9.6	4.4	2.0	510	normal	>10.0	D

I.N.S. = idiopathic nephrotic syndrome. Chr.N. = chronic glomerulonephritis. Purp. N. = purpuric nephritis. Explanation of categories A—D for steroid sensitivity, see in text.

resorted to it only if the histological picture of the kidney and the data regarding selectivity of proteinuria seemed to demand cytostatic therapy.

Material and Method

NS was diagnosed if

(1) proteinuria exceeded the value 5 g 24 hrs/1.73 m²;

(2) the concentration of total serum proteins was less than 5.0 g per 100 ml;

(3) the concentration of serum albumin was less than 2.5 g per 100 ml;

The clinical data regarding the material are assembled in Table I. As to the sensitivity to corticoids,

Category A consisted of patients whose symptoms disappeared on the administration of corticoids but who developed proteinuria as soon as treatment had cease.

Category B consisted of patients whose proteinuria improved significantly on the administration of steroids but did not fall below 0.5 to 0.1 g/24 hrs/1.73 m².

Category C: initial corticoid sensitivity of these patients turned into resistance after a number of months or years.

Category D: the members of this group were resistant to corticoids right from the onset of the disease.

Results

Renal biopsy and distribution of cases according to the histological findings

Explorative biopsy revealed no complications. Sections for light microscopic examination were stained with haematoxylin—eosin, PAS, Hart, and Jones' silver methenamine. Sainte-Maria's cold embedding technique was applied for immune-histological examinations. The 4 μ-thick sections were incubated with fluorescein isothiocyanate-bound human IgG IgM, complement, lipoprotein and

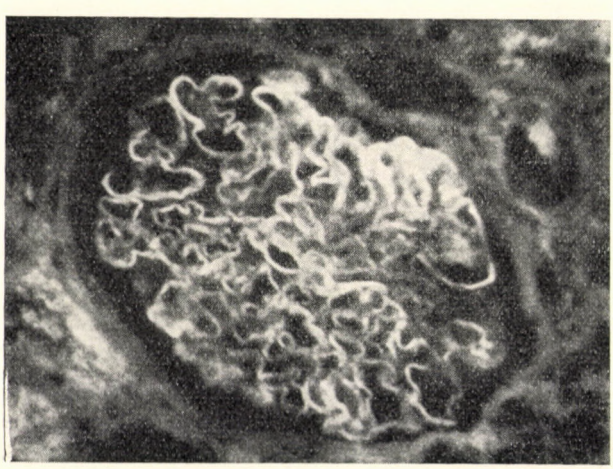

Fig. 1

albumin [25].* Inhibition was carried out in order to control the specificity of the procedure. Incubation with antihuman albumin served likewise the purpose of verifying the immunological process.

15. The basement membranes were practically outlined by the IgG in these cases (Fig. 1). A small amount of the substance, deposited in patches, was found in patient No. 3, while the arrangement was granular

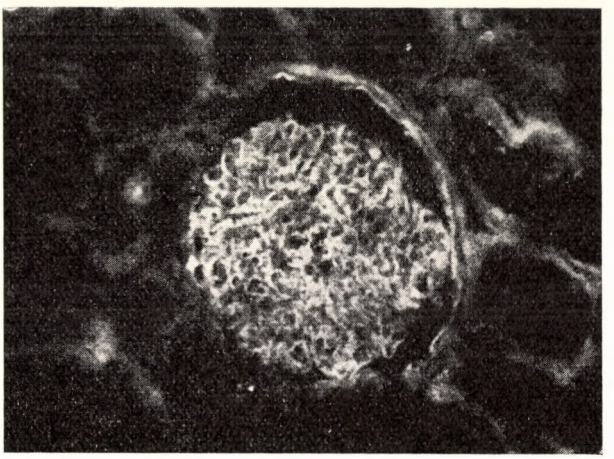

FIG. 2

Material for electron-microscopy was fixed in 2% osmium tetroxide adjusted to pH 7.2 by veronal acetate buffer and then embedded in araldite. Contrasting was performed with uranyl acetate and Karnovsky's lead oxide. An electron microscope, type SEM-3-1, was used.

With the exception of Case No. 11 (Table I), the presence of IgG was invariably demonstrated on the glomerular basement membrane by the immune-fluorescence method. The amount of IgG was variable, and its arrangement either linear [14] or granular [18, 26, 27, 32]. A large amount of linearly arranged IgG was found in patients Nos 1, 2, 5, 6, 13, and diffuse in patients Nos. 4 and 8 (Fig. 2). The amount of granular immune deposits was smaller and arranged patchwise in cases Nos 7, 9, 10, 12, 14. There seemed to be no correlation in morphology between the immune deposits and the therapeutical results. Arrangement of the complement was similar to that of IgG. IgM and fibrin were also in the glomeruli. Fibrin was rarely found on the basement membranes; it was more frequently situated between the endothelial and epithelial cells, a finding in harmony with the observations of MCCLUSKEY et al. [24]. In no case was albumin found in the glomeruli, and the inhibition test proved the

* The fluorescein isothiocyanate-bound antihuman sera were received from the Sylvana Chemical Co., New Jersey, N. J., and Messrs C. Heyman, Brussels.

Table II

Results of treatment

Patient	Haemoglobin g/100 ml		Leucocytes 1000/cu mm		Lymphocytes 1000/cu mm		Thrombocytes 1000/cu mm		Serum IgG mg per 100 ml		Serum IgM mg per 100 ml		Serum IgA mg per 100 ml		Mantoux test 1:1000/mm²			
															24 hrs		72 hrs	
No.	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
1	13.1	10.9	23.6	3.6	3.6	0.4	240	160	860	610					88	25	64	16
2	12.7	11.8	5.0	5.0	2.5	0.7	240	180	760	720					120	42	64	16
3	10.1	8.5	14.0	0.6	3.7	0.1	180	48	890	82	170	95	210	28	144	0	64	0
4	12.7	10.6	12.0	5.6	3.9	1.0	—	—	310	450					—	—	—	—
5	12.4	10.2	6.8	2.8	2.9	0.9	140	80	1020	510					220	63	170	25
6	13.7	9.0	8.0	3.4	2.9	0.7	220	110	770	530					100	64	100	16
7	12.8	10.9	12.0	5.1	2.8	1.0	230	160	415	560	115	43	150	77	370	150	220	25
8	13.1	11.8	9.0	2.2	2.9	0.4	180	70	560	510					110	80	100	16
9	14.1	12.2	11.0	2.0	3.3	0.6	180	76	480	230		77		32	170	48	130	20
10	15.3	12.2	8.0	2.8	2.4	0.6	210	87	340	240		119		88	100	64	80	0
11	13.1	12.1	7.5	2.2	2.4	0.4	240	110	350	370	150	36			660	54	150	25
12	15.2	13.2	6.2	3.8	1.0	0.2	160	160	1210	840	145	86	150	36	144	42	96	36
13	12.7	11.2	6.5	5.0	1.3	0.9	200	170	670	550					64	42	64	16
$\overline{X}$	13.2	11.2	8.4	3.4	2.7	0.6	200	118	660	470	165	76	170	52	175	52	100	16
N	13	13	13	13	13	13	12	12	13	13	5	6	3	5	12	12	12	12

1 = pretreatment figures

2 = lowest therapeutic values

specificity of the process. The cytoplasm of the tubular cells contained some lipoprotein.

Following the nomenclature of Ogg et al. [30] we classified the light-microscopic pictures as follows.

(1) *Minimal changes.* Light microscopy and immune-histology revealed no changes. Electron-microscopy showed a slight thickening of the basement membranes as also that of the podocytic foot processes. Changes in this category were not observed in our material.

(2) *Membraneous nephropathy* (referred to as *M* in the following and in the Figures). Thickening of the glomerular basement membranes was visible under the ligth microscope. Immune deposits were distinguishable on the basement membranes or in the mesangium. Ten of the cases belonged to this category. In three of them (Mchr. in Table III) the glomeruli displayed initial symptoms of chronic alterations; some of the glomerular loops adhered to

Table III

Clinical diganosis and renal biopsy
Selectivity index of proteinuria (S.I. = $C_{IgG} : C_{Albumin}$)
Gel filtration on Sephadex-G 200

No.	Diagnosis	Histology	S.I.	Gel filtration, %				Result; time of observation
				19S	7S	4.6S	Prealbumin	
1	I.N.S.	M.	0.05	3.4	7.5	80.9	8.2	A, 20 months
2	I.N.S.	Mchr.	0.12	—	—	—	—	A, 20 months
3	I.N.S.	M.	0.04	1.3	5.4	88.6	4.7	A, 10 months, relapse
4	I.N.S.	M.	0.01	—	—	—	—	A, 21 months
5	I.N.S.	M-P	0.08	—	—	—	—	A, 16 months
6	I.N.S.	M.	0.01	6.8	5.3	60.8	27.2	A, 16 months
7	I.N.S.	M.	0.06	0.5	7.5	72.5	19.5	A, 16 months
8	I.N.S.	Mchr.	0.08	7.6	3.8	87.4	1.2	A, 8 months
9	I.N.S.	Mchr.	2.20	10.6	18.2	71.9	1.2	B, 8 months
10	I.N.S.	M.	0.30	18.8	18.2	46.2	6.8	B, 8 months
11	Chr.N.	M.	0.10	0.9	5.3	72.1	21.7	C
12	Chr.N.	M-P	0.09	10.7	14.2	52.3	22.8	C
13	Chr.N.	M-P	0.14	2.8	30.6	46.1	20.5	C
14	Pupr.N.	SDGN.	0.21	2.9	3.6	48.4	45.1	C, spont. recov.
15	Purp.N.	SDGN.	0.60	1.2	3.4	49.5	45.9	C, spont. recov.

S.I. = selectivity index
Spont. recov. = subsequent spontaneous recovery
M = membraneous glomerulopathy
Mchr. = membraneous glomerulopathy with signs of impending chronicity
M-P = membrano-proliferative glomerulonephritis
SDGN = segmental diffuse glomerulonephritis
A, B, C = categories of result (see text)

Bowman's capsule which showed signs of incipient hyalinization, the number of argentophile fibres was increased and occasional hyalinized glomeruli were observed.

(3) *Proliferative glomerulonephritis.* The material contained 3 cases of membrano-proliferative glomerulonephritis. The glomeruli were considerably enlarged and rich in cells; proliferation of the endothelial cells was accompanied by a thickening of the glomerular basement membranes (Fig. 3). Immune fluorescence revealed the presence of IgG on the basement membranes and some lipoprotein in the epithelial cells.

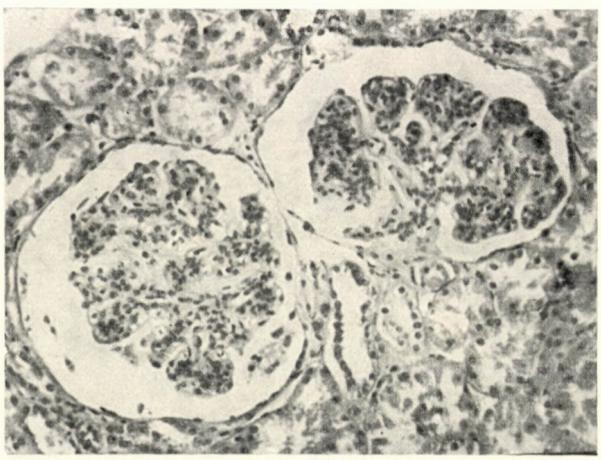

FIG. 3

(4) *Segmental diffuse glomerulonephritis* was found in 2 cases of purpuric nephritis (Fig. 4). Immune fluorescence revealed linearly arranged IgG in patient No. 15 (Fig. 5). Granules of fibrin and IgM were found on the basement membranes. Case No. 14 in which the IgG was granularly arranged, showed a much more serious clinical picture.

Histological findings are listed in Table III.

Selectivity of proteinuria

Urine samples were collected at 4° C. Total protein in serum and urine was determined by the biuret method. Urines with less than 1 g per 100 ml of protein were concentrated at 4° C by dialysis against 10% high molecular dextran. Fractionation of the proteins was done by electrophoresis and gel filtration.

Paper electrophoresis, immune electrophoresis and quantitative radial immunodiffusion [23] were employed in every case for the determination of albumin in IgG concentration and in some cases for that of

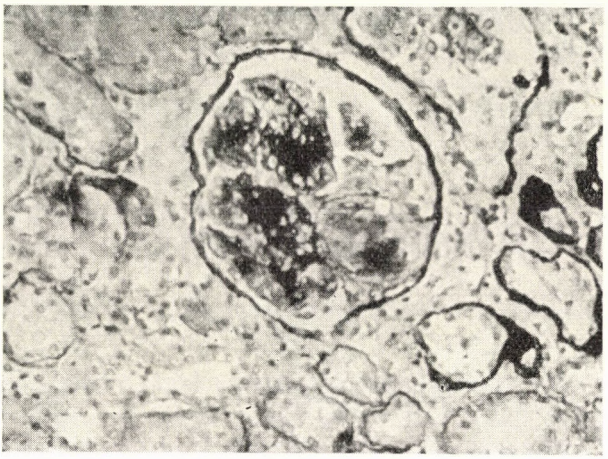

FIG. 4

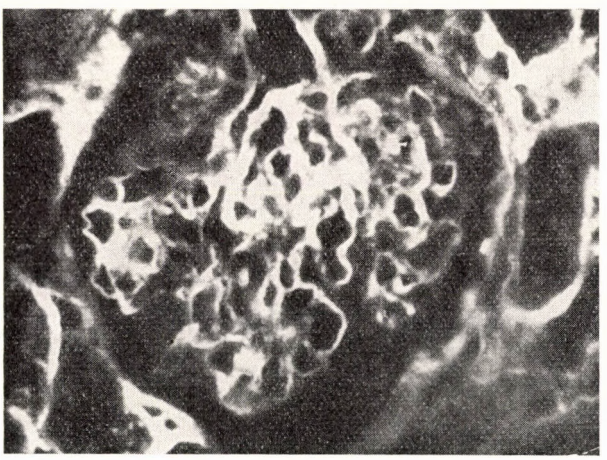

FIG. 5

IgM and IgA. concentration* Albumin- and IgG-clearance was calculated from the results of these tests methods, and computed then the index of selectivity (SI).

$$SI = C_{IgG} : C_{Albumin}.$$

We determined in 12 cases the distribution of urinary proteins on Sephadex-G 200 column [16]. The distribution of 17S, 9S, 4.6S and prealbumin was computed by planimetry.

Table III shows the selectivity indexes and the results of gel filtration.

Treatment

Treatment with cyclophosphamide + prednisolone was prescribed according to MONCRIEFF et al. [28]. The

* Anti-IgG, anti-IgA and anti-IgM sera were products of the Serum and Vaccine Institute, Praha. The mixed normal serum of Human, Budapest, served as standard.

initial daily doses were 0.3 to 0.5 mg/kg for prednisolone and 3.0 mg/kg for cyclophosphamide. The daily dose was increased to 5 and sometimes transitorily to 6 mg/kg after the first week. We tried to depress the leucocyte count below 5000 (keeping it possibly between 3000 and 4000) and to keep at the same time the number of thrombocytes above 100 000. Blood counts were checked at 2—3 day intervals, and after the treatment became effective and the individual dosage had been adjusted, i.e. after the 4th or 5th week—once every week. If it was effective, treatment was continued for 6 to 8 months. The patients stayed in the institute during the first 6 to 7 weeks of the therapy and were then treated as outpatients. The discharged children were allowed to lead a normal life and to attend school. In Cases Nos 11—15, treatment had no effect and was abandoned after six weeks.

Treatment and course of the disease were different from the other cases in Case No. 3. The condition of this child was poor, proteinuria and oedema had not responded to large doses of corticoids. Then azathioprine was prescribed for 7 days and 6 days later chlorambucil in doses of 0.8 mg/kg daily. On the 10th day of this treatment the child developed serious immune depletion; there appeared gangrenes in the mouth; the leucocyte count dropped to 600, the number of thrombocytes to 34,000; the concentration of IgG was 82 mg per 100 ml, IgA, 28 mg per 100 ml; IgM showed an approximately normal value (177 mg per 100 ml). Adequate therapy improved the patient's condition in two weeks, and the proteinuria had also disappeared. The urine was free from protein during the next 19 months without further medication, and the disease seemed to have completely ceased. A relapse ensued after 19 months. We performed a renal biopsy, determined the selectivity of proteinuria and instituted combined cyclophosphamide and prednisolone treatment. Complete remission was achieved in 6 days; the therapy, now in the fourth month, is still being continued.

I. Proteinuria and haematuria. Results are listed in Table III. The following categories were set up.

A. Complete remission. Proteinuria disappeared after 6 to 14 days, and the urine has been free from protein without drugs since the termination of the treatment. Patients Nos 1 to 8 belonged to this category.

B. Partial remission. The grave initial proteinuria dropped below 1.0 g/24 hrs/1.73 m^2 (Cases Nos 9 and 10). The condition had improved gradually in about 4 to 6 weeks.

C. No improvement. The NS of these patients (Nos 11 to 15) was accompanied by haematuria. Treatment was unsuccessful and was abandoned after 6 weeks. The condition of three patients (Nos 11, 12, 13) suffering from chronic glomerulonephritis showed no change during the subsequent two years. On the other hand, patients Nos 14 and 15 recovered 16 and 7 months, respectively, after the termination of

the treatment (no proteinuria and haematuria, normal renal function tests).

II. Blood counts. Initial and lowest values in the 3rd to 6th week of drug treatment are shown in Table II. These values increased normally in the further course of treatment even if the dosage of cyclophosphamide had not been reduced. In some cases it was necessary to give smaller doses or to interrupt the administration of cyclophosphamide for some time.

Treatment was interrupted for 6 to 10 days in 5 cases on account of thrombocytopenia, when the WBC also fell below 3000. Leucopenia with lymphopenia was a characteristic feature. We determined in every case that individual dosage which ensured a practically normal thrombocyte count and a lymphocyte count of about 1000. The treatment of patient No. 11 had to be interrupted on account of leucopenia without concomitant thrombocytopenia.

III. Concentration of serum immune globulins. The concentration of IgG was determined in all cases, that of IgM in 6, and that of IgA in 5 cases. Results yielded by HEREMANS' radial immunodiffusion [23] agreed with literary data [3, 10] and are listed in Table II. The IgG level amounted to about 500 mg per 100 ml against the normal 1000 mg. Compared to the initial hypogammaglobulinaemia, the value of 500 mg per 100 ml meant a rise in some cases. Minimal values were registered between the 4th and the 7th week of treatment, after which the blood level of immune globulins moved sligthtly upward, a phenomenon well known in literature.

IV. Mantoux test. All patients had been inoculated with BCG during the first week of extrauterine life. In no case was tuberculosis mentioned in the history nor did the X-ray pictures show such signs. Of a freshly prepared 1 : 1000 solution of old tuberculin, 0.1 ml was injected into the skin above the scapula. The size of the infiltrated area was measured at 24 and 72 hours. Results are shown in Table II. The reduced intensity of the reaction, while observable after 24 hrs already, was especially marked on the third day. Quickly disappearing reactions of reduced intensity illustrate the well-known effect of the treatment in respect of delayed hypersensitivity [40, 45]. Table II does not show that, between the 3rd and the 6th week after the treatment, reaction intensity was usually like that before the treatment (in 3 cases it was considerably increased). Hyperergic reactions in these 3 patients returned to normal after a number of months.

V. Chromosomes. Cytostatic drugs are known to induce chromosomal aberrations. There are several reports on the mutagenic properties of cyclophosphamide [15, 33, 35, 36, 38, 39]. Although no malignancy has so far been described as a consequence of cyclophosphamide treatment and despite the fact that animal experiments have shown this compound to be less cancerogenic than chlorambucil [38], opinions are divided as to whether long-term treatment may neverthe-

less involve the risk of tumour development [8, 34, 46]. In nine of our cases the pretreatment incidence of chromosomal breaks amounted to less than 2% (between 0 and 6%). It rose to 18% between the 2nd and 4th month of cyclophosphamide therapy, still amounted to 16% 2 to 4 weeks after its termination, and dropped to 5—7% after the lapse of another 2 to 4 months. Further examination of our patients from this aspect is now in progress [6]. Observations made so far make it in any case evident that the institution of cytostatic therapy must very reliably be justified.

VI. Toxic side effects. Literature contains several reports on the various side effects of cyclophosphamide [8, 12, 37, 44, 45]. In the present material, the following side effects were observed.

(1) Complete alopecia in four cases; lost hair was replaced in the further course of treatment.

(2) Sterile haemorrhagic cystitis in three cases. Treatment was interrupted for 8—12 days, and the condition did not reappear on the resumption of therapy. Cystitis was accompanied by proteinuria; the original composition of the urinary proteins was changed, and much gamma globulin, further prealbumin appeared in the urine also in cases of highly selective proteinuria. Cessation of proteinuria went hand in hand with the subsidence of the inflammatory changes.

(3) Other side effects such as stomatitis, thrush, gingivitis, enteritis or grave infections were not observed. Our patients had already had measles and chickenpox. No antibiotics were administered.

Discussion

Fifteen cases of steroid-resistant or dependent NS have been treated with a combination of cyclophosphamide and prednisolone. Renal damage was serious in all cases; none of the patients belonged to the minimal changes category.

No correlation appeared to exist between the therapeutic results and the light-microscopic, electron-microscopic and immune histological pictures. Two factors seemed to determine the success or failure of treatment, the degree of haematuria and the selectivity of proteinuria. Treatment was successful of the 15 cases in 10. They were diagnosed as idiopathic nephrotic syndrome; these 10 patients had no haematuria. The degree of the selectivity of proteinuria seemed to influence the result. Patients with good selectivity responded readily even in cases of membraneous glomerulonephritis with signs of chronicity (cases Nos 2 and 8) and in a case (No. 5) of membranoproliferative glomerulonephritis, while remission was only partial in patients Nos 9 and 10 whose selectivity was unsatisfactory. Selectivity and therapeutic results were thus correlated in these cases, a repeatedly described phenomenon [11, 22, 41].

The other 5 patients had haematuria: a 6-week treatment failed to

reduce the degree of either the proteinuria or the haematuria. Chronic glomerulonephritis was diagnosed in three of these patients whose proteinuria selectivity was not bad. The selectivity index furnished in these cases no prognostic information.

Changes in the concentration of serum immune globulins and the decrease of delayed hypersensitivity as shown by the Mantoux test were in harmony with literary data. Observations in connection with chromosomal aberrations made in the course of treatment as also investigations still in progress in this respect prove that the use of cyclophosphamide involves grave risks and should, therefore, be applied with great circumspection.

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