SERUM PROTEINS IN MONGOLISM

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IN recent years electrophoretic methods for the analysis of the serum proteins have been extensively and fruitfully applied to studies of diseases of the nervous system (Volk, Saifer, Rabiner and Oreskes, 1955; Volk, Saifer, Rabiner and Hinterbuchner, 1956; Press, 1956).

Investigations of the serum proteins in mongolism by classical chemical methods failed to establish significant abnormalities (Benda, 1947). However, the possibility that a protein profile specific to mongolism might be demonstrable by the more sensitive electrophoretic techniques prompted us to reinvestigate the matter.

CLINICAL GROUPS

The investigation was carried out on 36 children with mongolism, each being paired with a mentally defective child as control. The pairs were matched for age, weight and diet, and drawn wherever possible from the same wards. Eighteen of the pairs fell in the age group 2–6 years and 18 in the age group 6–10 years. The mean I.Q. of the mongoloid children was 34, range 5–67, while that of the controls was 31, range 3–57.

The classification of the patients is shown in Table I. "Unclassified" here means that the mental defect could not be attributed to any specific cause. Five of the patients in this sub-group showed psychotic features. As far as could be ascertained all patients were free from infection.

TABLE I
Classification of Cases

Mongolism	36
Cerebral palsy	6
Birth injury	4
Tuberculous meningitis	1
Icterus gravis neonatorum	2
Microcephaly	4
Epilepsy	2
Unclassified	17

METHODS

Collection of Samples

Capillary blood was used throughout. About 0·2 ml. of blood was obtained from a finger prick and allowed to clot in a $\frac{3}{8}$ in. tube. Half an hour after clotting had taken place the serum was separated by centrifuging for 10 minutes at 2,500 rev./min.

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Electrophoresis on Paper

The experiments were carried out within half an hour of the separation of the serum by the horizontal open strip technique (Grassmann and Hannig, 1952). 0·01 ml. of serum was applied by a 1¼ inch microscope slide to strips of Whatman No. 1 paper and run for 5 hours at 2° C. in barbiturate buffer pH 8·6, ionic strength 0·05, at a current of 0·3 mA/cm. and a potential of approximately 17 V/cm. length. To minimize evaporation the tank was fitted with a rubber gasket.

At the end of the run the proteins were denatured by heating in an oven for 30 minutes at 110–120° C. and stained in a 0·5 per cent. solution of light green S.F. (Hopkin and Williams Ltd.) in water containing 25 per cent. ethanol and 5 per cent acetic acid (Dangerfield and Smith, 1955). After clearing of the background with 2 per cent. acetic acid and drying, the strips were made translucent by immersion in a mixture of 20 vol. liquid paraffin, 12 xylene, 10 bromonaphthalene and 1 per cent. Tween 80 special grade (Honeywill and Stein Ltd.), (Rees and Laurence, 1955). After surface drying with filter paper the strips were scanned with an EEL scanner (Evans Electroselenium Ltd.) using a Chance OY4 yellow filter.

From experiments with Armour bovine serum albumin and γ globulin it was found that in our experimental conditions albumin absorbed 1·3 times as much dye as globulin. This was corrected for in the computation of the concentration of the serum protein fractions. Deviations from Beer's law were corrected for by the method of Latner (1955). No correction was made for the albumin tail. Samples from mongols and their matched controls were without exception taken together through the entire estimation. In this way conditions for electrophoresis, protein denaturation, staining and scanning were identical for each pair.

Chemical Methods

Total proteins were estimated by precipitation from a 1 : 500 dilution of serum in saline with an equal volume of 10 per cent. trichloracetic acid, followed by digestion with a 10 per cent. solution of selenium in concentrated sulphuric acid for half an hour, and Nesslerization.

Zinc sulphate and thymol turbidity tests were carried out according to Kunkel (1947) and Maclagan (1944) and the turbidities read against Mestrezet standards (Baird and Tatlock Ltd.).

Results

The mean values for the protein fractions in the sera of the mongols and controls are shown in Table II.

While the total proteins are almost identical in both groups, the albumin is significantly lowered and the γ globulin raised in patients with mongolism. The lowering of the serum albumin is entirely due to the highly significant difference in the 6–10 year group; there is no significant difference in the age group 2–6 years.

When the two age-groups are compared (Table III) it is found that the α_1, α_2 and β globulin, but not the γ globulin, are lower in the 6–10 year age group, both in controls and in patients with mongolism. In the former, but not in the latter, the serum albumin is higher in the 6–10 year group.

Experiments with the zinc sulphate and thymol turbidity tests (Table IV) are fully consistent with the electrophoretic measurements, higher turbidity readings, although still within the normal range, being found in patients with

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TABLE II

Serum Protein Fractions Determined by Paper Electrophoresis and Total Proteins in the Sera of Mongoloid Children and of Other Mentally Retarded Children as Controls. Significant Differences ($P<0.05$) are Shown in Heavy Type

Protein Fraction	Mongols			Controls			Significance of Differences		
	N	Mean Percentage of T.P.	S.E.	N	Mean Percentage of T.P.	S.E.	Δ		P
bumin:									
Age 2–10 years	36	48·3	0·77	36	53·4	0·84	5·1	>3·460	<0·001
Age 2–6 years	18	46·9	1·85	18	50·3	1·15	3·4	<1·684	>0·1
Age 6–10 years	18	49·6	1·04	18	56·4	0·92	6·8	>3·646	<0·001
α_1 globulin:									
Age 2–10 years	36	6·1	0·34	36	6·2	0·23	0·1	<1·671	>0·1
Age 2–6 years	18	6·7	0·29	18	6·7	0·27	0·0	<1·684	>0·1
Age 6–10 years	18	5·5	0·35	18	5·6	0·34	0·1	<1·684	>0·1
α_2 globulin:									
Age 2–10 years	36	10·9	0·29	36	11·1	0·31	0·2	<1·671	>0·1
Age 2–6 years	18	11·7	0·44	18	11·9	0·34	0·2	<1·684	>0·1
Age 6–10 years	18	10·1	0·22	18	10·3	0·43	0·2	<1·684	>0·1
β globulin:									
Age 2–10 years	36	13·1	0·28	36	12·6	0·27	0·5	<1·671	>0·1
Age 2–6 years	18	13·6	0·39	18	13·5	0·30	0·1	<1·684	>0·1
Age 6–10 years	18	12·6	0·23	18	11·7	0·33	0·9	>2·042	<0·05
γ globulin:									
Age 2–10 years	36	21·7	0·75	36	16·7	0·53	5·0	>3·460	<0·001
Age 2–6 years	18	21·1	1·28	18	17·5	0·84	3·6	>3·646	<0·001
Age 6–10 years	18	22·3	0·85	18	16·0	0·50	6·3	>3·646	<0·001
Total Proteins (g./100 ml.):	**N**	**Protein level**	**S.E.**	**N**	**Protein level**	**S.E.**	**Δ**	**t**	**P**
Age 2–10 years	35	6·58	0·09	36	6·59	0·07	0·01	<1·671	>0·1
Age 2–6 years	18	6·58	0·12	18	6·65	0·12	0·07	<1·684	>0·1
Age 6–10 years	17	6·58	0·14	18	6·53	0·08	0·05	<1·684	>0·1

TABLE III

Comparison of Serum Protein Fractions Determined by Electrophoresis, and Total Proteins in Two Age Groups of Mongoloid Children and Other Mentally Retarded Children as Controls. N=18 except where Otherwise Stated. Significant Differences ($P<0.05$) are Shown in Heavy Type

Protein Fractions	Age 2–6 Years		Age 6–10 Years		Significance of Differences		
	Mean Percentage of T.P.	S.E.	Mean Percentage of T.P.	S.E.	Δ	t	P
Albumin:							
Mongols	46·9	1·85	49·6	1·04	2·7	<1·684	>0·1
Controls	50·3	1·15	56·4	0·92	6·1	>3·646	<0·001
α_1 globulin:							
Mongols	6·7	0·29	5·5	0·35	−1·2	2·457	<0·02
Controls	6·7	0·27	5·6	0·34	−1·1	2·457	<0·02
α_2 globulin:							
Mongols	11·7	0·44	10·1	0·22	−1·6	>2·750	<0·01
Controls	11·9	0·34	10·3	0·43	−1·6	>2·750	<0·01
β globulin:							
Mongols	13·6	0·39	12·6	0·23	−1·0	>2·042	<0·05
Controls	13·5	0·30	11·7	0·33	−1·8	>3·646	<0·001
γ globulin:							
Mongols	21·1	1·28	22·3	0·85	+1·2	<1·684	>0·1
Controls	17·5	0·84	16·0	0·50	−1·5	<1·684	>0·1
Total Proteins (g./100 ml.):	**Protein level**	**S.E.**	**Protein level**	**S.E.**	**Δ**	**t**	**P**
Mongols	6·58	0·12	6·58*	0·14	—	<1·697	>0·1
Controls	6·65	0·12	6·53	0·08	−0·12	<1·697	>0·1

TABLE IV

Zinc Sulphate and Thymol Turbidity Reactions in the Sera of Mongoloid Children and of Other Mentally Retarded Children as Controls

Turbidity Reaction	Mongols			Controls			Significance of Differences		
	N	Mean (Mestrezet Units)	S.E.	N	Mean (Mestrezet Units)	S.E.	Δ	t	P
Zinc sulphate	20	2·50	0·18	20	1·40	0·09	1·10	>3·551	<0·001
Thymol	20	1·45	0·28	20	0·83	0·11	0·62	>3·551	<0·001

* N=17.

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mongolism. This one would expect as it is particularly the level of the γ globulin that is reflected in these precipitation reactions.

Discussion

Owing to the ease with which small differences, particularly in staining and scanning techniques, can affect the quantitative analysis of serum proteins by paper electrophoresis (Martin and Franglen, 1954; Schulz and Holdcroft, 1956), great caution must be exercised in comparing results obtained in different laboratories. Bearing this in mind the data for our controls in the 6–10 year age group are in satisfactory agreement with data for normal children in the literature (Caspari, Negri and Sticca, 1953; Plückthun, 1953).

The changes with age in the protein distribution are of some interest, particularly in the control group. In normal children the serum albumin and the α and β globulin reach their adult levels at an age of about 2 years (Caspari, Negri and Sticca, 1953; Orlandini, Sass-Kortsak and Ebbs, 1955; Plückthun, 1953). The significant differences in the levels of these proteins between the 2–6 and 6–10 year olds (Table III) show that the protein pattern has not yet become stabilized in the 2–6 year group. In mentally defective children adult levels are thus reached at a much later age than in normal children. This is not surprising as the physiological age of many of the children was evidently well below their chronological age. The mongoloid children are even more retarded with respect to their serum protein pattern, particularly the rise in the serum-albumin level with age, than the controls. This at least partly accounts for the fact that differences between mongols and controls in the albumin and β globulin are significant in the 6–10 year age group but not significant in the 2–6 year group. The γ globulin, in contrast, reaches stable levels in both controls and mongols if anything earlier than in normal children.

The protein profile in mongolism with its raised γ globulin and lowered albumin is unlike any observed so far in patients with other lesions of the central nervous system (Volk, Saifer, Rabiner and Hinterbuchner, 1956; Press, 1956). It resembles that found as an aftermath of an infection, in chronic infections, in conditions of suboptimal liver function and in some cases of leukaemia (Flynn, 1954; Martin and Franglen, 1954; Vera and Roche, 1956). It has also been suggested (Lewis and Page, 1956; Page, Lewis and Gilbert, 1956) that genetic factors may affect the relative proportion of albumin and γ globulin.

There was no detectable difference in the general state of health of our patients with mongolism and controls, so that it is improbable that a greater prevalence of infections in the former at the time of test could account for the observed differences. The raised level of the γ globulin is of particular interest. According to modern views γ globulin is complex biologically and physico-chemically. It is synthesized in several types of cell in the bone marrow, spleen and lymph nodes (Askonas, Humphrey and Porter, 1956). A raised level is associated with hyperactivity of the reticulo-endothelial and/or haematopoietic systems. The high γ globulin would therefore fit in well with the "shift to the left" in the polymorphonuclear leucocytes (Turpin and Bernyer, 1947; Shapiro, 1949; Mittwoch, 1956) and with the increased tendency to leukaemia postulated by Krivit and Good (1956) and Merrit and Harris (1956). According to these authors the severe injury to the foetus in the first 6–7 weeks which results in mongolism may affect the bone marrow and haematopoietic system as well as any of the other anatomic structures undergoing rapid differentiation at that time, e.g. brain, heart and skeleton. This might well result in a "shift to the left", and if the injury is exceptionally severe, in leukaemia. Speculation on

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how far the observed protein pattern is caused by suboptimal liver function and how far indirectly by injury to the haematopoietic system appears profitless at this stage. It is hoped that experimental work now in progress on other aspects of metabolism in mongoloid children will throw light on this matter.

Summary

1. The serum albumin was decreased and the serum γ globulin increased in a group of 36 mongoloid children compared to a matched group of controls. There was no significant difference in the total proteins, α_1, α_2, and β globulins.

2. Higher turbidity readings, though still within the normal range were also found for the children with mongolism in the zinc sulphate and thymol turbidity tests.

3. When a comparison was made between the age groups 2–6 years and 6–10 years significant differences were observed in all the globulins except the γ globulin both in the mongoloid children and in the controls. In the latter, but not in the former group, there was also a significant difference in the serum albumin.

4. The possible significance of these findings is briefly discussed.

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