A NOTE ON THE ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN ACUTE HAEMOLYTIC ANAEMIA OF RATS *

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Received on December 31, 1958

Abstract

Phenylhydrazine in 12 mg./kg. dose induces severe hemolytic anæmia associated with pathological involvement of liver, spleen and other organs, simulating those observed in acute experimental malaria. Since the etiological factors and the sequence of events involved in the hæmolysis are different, a study of biochemical changes in this condition has been carried out and compared with those observed in other toxic and hæmolytic states.

Electrophoretic studies on rat serum proteins in phenyl-hydrazine induced hæmolytic anæmia of rats indicate practically no alteration in total serum protein but appreciable increase in γ globulin and a slight decrease in albumin. No marked changes in α_1, α_2 and β globulins and no new components have been observed. Mobilities do not show any change in this pathological state.

Since no infective or antigenic stimulation is involved, no explanation can be offered for the large increase in the γ globulin fraction observed in the hæmolytic anæmia.

Hæmolytic anaemia is observed in a variety of clinical conditions in which there is an excessive destruction of red blood cells. This haemolysis may be associated with intrinsic abnormality of red cells or to the injury of fundamentally normal erythrocytes. Physical, bacterial, parasitic, allergic, chemical and immunologic agents are some of the important extrinsic factors responsible for the haemolysis of the latter type of cells. The harmful effects of haemolysis result not only from anaemia, but also from the various types of injury brought about by agglutinated red cells in the capillaries of various organs and by the liberated hæmoglobin products, the resulting damage affecting the liver, kidney and other organs.

Phenylhydrazine induced anæmia has been utilized by many investigators as a suitable model for therapeutic evaluation of haematopoietic activity of drugs, since it simulates closely the changes observed in other heamolytic anaemias.

In our previous studies on the blood and tissue regeneration in haemolytic

* Forms part of thesis for which Mrs. V. V. Chiplunkar has been awarded the Ph.D. degree of the Bombay University.

anaemia associated with liver injury, phenyl-hydrazine in a dose of 12 mg./100 g. body weight of rats has been used. At this dosage it has been shown that pathological involment of liver, spleen and other organs closely simulate those in acute malaria.[1] Detail characterization of anaemia also depicts a similar trend.

But, severe haemolysis in malarial infection is only a late manifestation. During the evolution of the disease process pathological involment of organs and associated functional derangements are observed prior to severe haemolysis while with phenylhydrazine the process of haemolysis is a fairly rapid one and the involvement of the organs is either concomitant or develop after haemolysis. Since the etiological factors and the sequence of events in the haemolysis are different, it was considered desirable to study the biochemical alterations in the phenylhydrazine anaemia and compare it with the well-known changes observed in other haemolytic conditions.

With this object, serum protein changes in phenylhydrazine anaemia have been investigated and the results are reported in this note.

Experimental

The selection of animals and the procedure involved to produce acute haemolytic anaemia associated with liver injury has been described earlier.[1] Before and at varying intervals after phenylhdrazine (12 mg. per 100 g. body weight) administration, rats were sacrificed and the blood was collected from the heart, allowed to clot, centrifuged and serum separated. Michaelis buffer[2] of 8.6 pH ionic strength $\mu = 0.1$, has been employed for the electrophoresis of serum in all the experiments. After diluting with two parts of veronal-acetate buffer, the serum was dialysed at $0-4^{\circ}C$ for 48 hours, against two or three changes for buffer and finally against 250 ml. for a period of 24 hours by employing commercial cellophane tubing. After dialysis, the protein and the buffer solutions were equlibriated for 60 minutes by surrounding with water at the room temperature and then deaerated for 30 minutes. The serum was subjected to electrophoresis in Kern's micro-electrophoretic apparatus.[3] The conductivity of the buffer was also determined for calculating mobilities of the various protein fractions. The initial and final temperatures in the electrophoretic cells were noted for mobility corrections.

Mobility measurements:—The mobility of the normal and pathological protein fractions were calculated from the descending limb of the channel by Longsworth's method.[4]

By convention the mobility is given a positive sign if the protein migrates towards anode. The experiments were conducted at the laboratory temperature of $24 \pm 2^{\circ}C$. All the mobilities have been corrected to $25^{\circ}C$ by the procedure given by Johnson and Shooter.[5]

RESULTS

The interferometric patterns obtained for the ascendinding and descending limbs of the channel for serum proteins under normal and pathological conditions are given in Plate I. In this pattern each protein fraction shows a characteristic set of symmetrical fringes. Labhart and Stamb's[6] method has been employed for calculating the serum fractions. The total protein concentration has been determined by multiplying the number of fringes with the cell constant as indicated by Longsworth and MacInnes.[7] The total protein is also determined by micro-kjeldahl method in some cases to check up the cell constant.

TABLE I

The distribution of protein fractions in normal and pathological rat serum at 25° C in Michaelis barbiturate-acetate buffer of pH = 8.6 ionic strength $\mu = 0.1$ under potential gradient of 4.0 volts/cm.

No.	Days after phenylhydrazine administration		Total serum protein concentration g./100 ml.	Percentage Composition				
				Albumin	a_1	a_2	β	γ
1.	0	a*	4.161	62.24	5.68	8.01	14.60	9.61
		d†	4.096	63.14	5.11	8.02	14.68	9.04
2.	1	a	4.521	53.07	6.12	8.67	15.31	16.84
		d	4.403	56.63	5.61	9.70	13.77	14.29
3.	4	a	4.456	53.02	4.94	8.07	17.20	15.22
		d	4.359	58.31	4.61	8.18	14.16	14.32
4.	7	a	3.771	53.58	6.12	9.69	15.31	15.31
		d	3.770	60.71	5.10	8.16	13.26	12.76
5.	12	a	4.161	55.73	5.73	8.85	15.63	14.06
		d	4.161	58.09	5.05	9.60	15.16	12.13
6.	20	a	4.743	52.98	4.85	8.65	15.65	17.84
		d	4.548	59.91	5.08	9.14	22.77	13.20
7.	30	a	4.432	52.60	5.21	9.37	16.66	16.14
		d	4.356	58.76	5.15	9.28	14.43	12.37
8.	45	a	4.231	53.64	5.21	8.33	14.06	18.75
		d	4.325	55.16	5.15	9.28	12.88	17.52
Days after CCl_4 administration								
9.	2	a	2.423	53.86	6.16	8.72	16.92	14.87
		d	2.516	50.52	8.76	11.85	15.46	13.40

* ascending † descending

Table I gives the results of normal and pathological sera. The related mobility measurements are given in Table II. Measurements have been made on ascending and descending limbs, in conformity with the practice and literature.

TABLE II

Mobility ($\mu \times 10^5$) of protein fractions in normal and pathological rat serum at 25°C in Michaelis barbiturate-acetate buffer of pH = 8.6 ionic strength $\mu = 0.1$. under potential gradient of 4.0 volts/cm.

No.	Days after phenylhydrazine administration		Mobilites x 10^5cm.^{2} sec.$^{-1}$ volt^{-1} 25°				
			Albumin	a_1	a_2	β	γ
1.	0	a*	12.95	10.64	8.20	5.36	2.25
		d†	12.12	9.83	8.24	5.34	2.77
2.	1	a	18.86	16.20	14.09	11.14	7.22
		d	17.66	14.99	13.14	10.40	7.12
3.	4	a	13.30	10.94	8.10	4.81	1.78
		d	12.39	10.02	8.02	5.01	2.02
4.	7	a	13.51	10.86	8.78	5.72	3.20
		d	14.23	12.55	9.69	5.79	2.30
5.	12	a	13.50	11.48	8.98	5.48	2.27
		d	12.62	10.55	8.62	5.11	2.62
6.	20	a	15.61	13.34	10.98	7.34	3.43
		d	14.22	11.34	9.21	6.66	4.03
7.	30	a	16.87	14.62	11.87	8.32	3.79
		d	15.67	13.20	11.09	8.01	4.79
8.	45	a	14.88	12.11	9.34	6.26	2.76
		d	13.60	10.95	9.04	5.98	3.12

* ascending † descending

It can be seen from Plate I, Fig. I that ascending limb gives sharper boundaries than the descending one. Table I indicates practically no change in total serum protein but appreciable increase in γ globulin after phenylhydrazine administration, and this increase almost persisted till the end of 30 days. A slight decrease in albumin is evident. Besides these, there are no other marked changes in α_1, α_2 and β globulins, and no new component shows its presence in this syndrome.

The mobilities do not show any change in the pathological state and the minor differences in different experiments show the scattering in the values in all

experiments, and render it essential to carry out electrophoresis under rigidly fixed conditions.[8]

A comparative analysis of serum protein changes induced by carbontetrachloride (1.5 ml. per kg. body weight), a chemical known to cause liver injury is also shown in Tables I and II.

DISCUSSION

The nature of the serum protein changes occurring in some other diseases associated with haemolysis or/and liver injury is presented in Table III.

TABLE III

Nature of the serum protein changes occuring in other diseases, associated with haemolysis and/or liver injury

Sr. No.	Name of the deseases	Total proteins	Albumin	Globulins				References
				α_1	α_2	β	γ	
1.	Babesia	—	—					Gills *et al.* Ann. Trop. Med. Parsitol., 1953, **47**, 426
2.	Malaria	—	—				+	Thesis (Bombay University) Ph.D., by Rama Rao, 129
3.	Cirrhosis of the liver	—	—	N	N	N	+	Agarval *et al.* J. Indian Med. Assoc., 1957, **29**, 387
4.	Arsenic	—	—	N	N	N	+	Advances in internal medicine, 1950, **4**, 329
5.	Phenylhydrazine	N	—	N	N	N	+	
6.	Carbon tetrachloride	N	—	N	N	N	+	

— =decrease + =increase N =no appreciable change

Hypoproteinaemia, reduction in albumin content and increase of γ globulin appears to be the general pattern. The pathological involvement of the liver seems to be a common factor in all these diseases. Decrease in plasma protein concentration and marked loss of albumin is also known to occur by mere protein depletion as during starvation. The limited food intake and the interference with their synthesis in the liver probably explain the biochemical alteration of protein and albumin reduction in these diseases. In phenylhydrazine haemolytic anaemia, though liver exhibits centrilobular necrosis,[1] the serum protein alteration

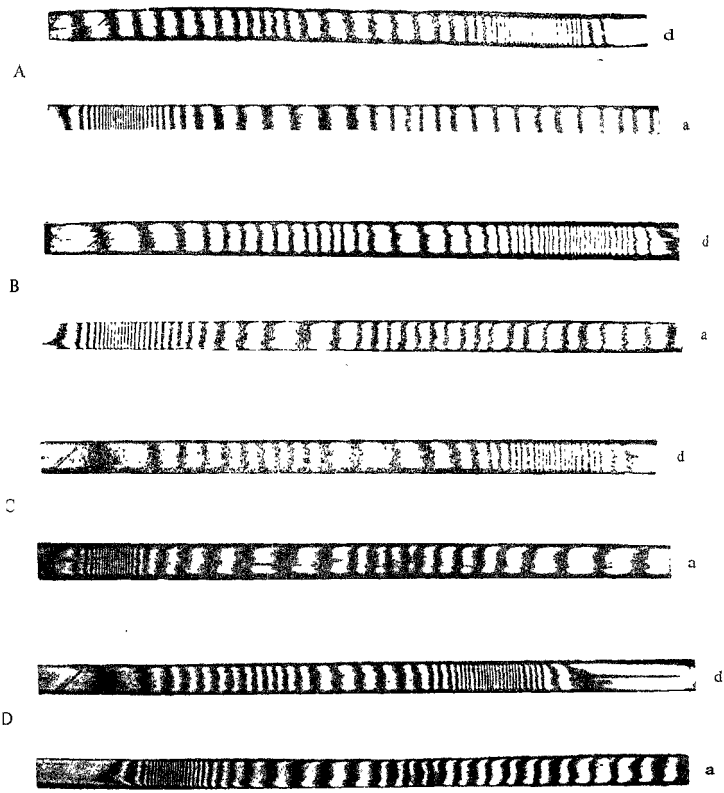

PLATE I

Electrophoretic pattern of rat blood sera in Michaelis buffer (pH 8.6) at 25° after 1 hour

A—Normal
B—Pathological sera after 4 days
C—Pathological sera after 8 days
D—Pathological sera after 30 days
d—descending limb a—ascending limb

is neglible while the albumin reduction is only of a slight degree. The increased breakdown of the haemoglobin and the resulting increased protein concentration in the body nitrogen pool may have compensated the reduction that should have occurred due to liver dysfunction.

Since no infective or antigenic reaction of the tissue is involved, the large increase in the γ globulin fraction noticed in the phenylhydrazine haemolytic anaemia is difficult to interpret.

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