COAGULATION COMPONENTS IN EXPERIMENTAL AND CLINICAL TUBERCULOSIS

By

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The whole blood and plasma clotting times, the fibrinogen content and prothrombin determination by one stage and two stage methods have been carried out in experimental and clinical tuberculosis. Acute progressive tuberculosis invariably resulting in the death of the animals was induced in the guineapigs by virulent human tubercle bacilli, H_{37} Rv and the coagulation components were estimated at the height of infection. Female patients in the Sanitoria, with wide spread pulmonary lesions, exhibiting acid fast bacilli in the sputum were the clinical cases studied. Healthy females of similar age group provided the normal data for comparative analysis.

Accelarated clotting process with diminished whole blood coagulation and calcium clotting times, a statistically significant increase in the fibrinogen content and decrease of prothrombin concentration were observed in acute guineapig tuberculosis.

In the chronic tuberculosis of female patients, while the biochemical derangements were similar to those observed in the acute guineapig tuberculosis, i.e. fibrinogen elevation and prothrombin diminution, the whole blood and calcium clotting times were also increased.

The possibility of utilizing the fibrinogen level of the blood as a measure of the extent and the spread of tubercular lesions and the implications of the observed changes of the other coagulation components in inducing haemmorrhagic diathesis are discussed.

The concept that blood coagulation comes into operation by the formation of a clot, only when bleeding due to injury of the blood vessels occurs is now giving place to a wider physiological role for coagulation. Continnous formation of fibrin within the normal circulatory vessels, the deposition of fibrin on the inner surface of the endothelium of blood vessels (Luscher, 1956) and its dissolution by anticoagulant factors, normally present in the plasma (Astrup, 1956) are considered to be a regular cyclic phenomena observed in all healthy individuals. The alterations in the coagulant and anticoagulant factors disturbs the dynamic equilibrium and favours either capillary damage and bleeding or formation of intravascular thrombi (Duguid, 1955).

While the major components involved in coagulation process and some of their precursors are well known, many new factors, which play a vital role in the clotting mechanisms are being isolated from the blood. In all conditions associated with coagulation defects, an analysis of these individual factors will greatly assist in formulating the correct therapy besides giving the biochemical basis for the disease.

The major components which come into operation at the terminal stages of clotting are fibrinogen, prothrombin, thromboplastin and calcium besides factors V and VII. The precursors of fibrinogen and prothrombin, their chemical nature, synthesis and mode of action are still controvertial topics. Coagulation process involves the synthesis of all these factors in the correct proportion and variations in any one of them, if it exceeds the adjustment limit is sure to result in a pathological manifestation.

Most of these coagulation components and the enzymes involved in the clotting reaction are protein in nature and conditions which interfere with this synthesis would naturally affect some part of this chain reaction. Though the end results might be similar, e.g. prolonged clotting time and bleeding or accellaration of clotting and thrombin formation, the causative components might be different. Since the clinical manifestations appear only when compensatory mechanisms fail, it will be difficult by mere clinical syndrome, to get an inkling of the nature of the changes leading to this final outcome and to adopt suitable therapeutic measures.

Tuberculosis in the fulminating type, is an acute inflammatory condition associated with tissue destruction and depressed functioning of all vital organs. Blood stained sputum, indicative of bleeding from the capillaries is a frequent accompaniment. Whether this haemmorhage is merely one due to capillary damage or reflects more vital changes in the blood coagulation components needs elucidation.

In the chronic fibrocaseous type of tuberculosis, though frank hemoptysis is thought to be due to the opening up of bigger blood vessels, the quantitative alterations of the coagulation components have not been so far defined. The fibrinolytic activity of blood in experimental and clinical tuberculosis have been reported earlier (Chiplunkar and Sirsi, 1962).

The present investigations have been undertaken to obtain information on the variations in other coagulation components during these disorders.

Experimental tuberculosis in guineapigs and chronic but active tuberculosis in adult famales admitted to the Sanitoria have been the subjects of study.

METHODS

Experimental guineapig tuberculosis.—Tuberculin negative guineapigs were infected with a virulent strain of *Mycobacterium tuberculosis* ($H_{37}R_v$) The dose given was such as to cause progressive disease invariably ending in death of the animals in about 4 months. Details about the time and mode of collection of blood samples, the extent of lesions observed at the period etc. have been described earlier (Chiplunkar and Sirsi, 1962).

Adult guineapigs, weighing 300-500 g were housed in separate cages and maintained on the standard laboratory diet consisting of leafy vegetables (40%), wheat bran (20%), and soaked Bengal gram (40%). They were tuberculin tested by intradermal injection of 0.1 of 1/10 dilution of O.T (human)) and all were found to be negative prior to infection.

A group of ten animals were taken for experimental study. Values were obtained for each animal prior to infection and at the required periods after infection with *M. tuberculosis.*

Tuberculin testing with 0.1 ml O.T., of 1 : 1000 dilution was done, four weeks after infection. The weights of the animals were recorded once a fortnight. When rapid loss in weight and positive tuberculin reaction indicated the spread of the disease, blood was withdrawn from heart puncture and the animal sacrifieed to know the extent of lesions in the body.

Infection : $H_{37}R_v$ strain of *M. tuberculosis*, grown for two weeks as a surface culture on Youmans media was emulsified in saline so as to contain 10 mg/ml and 0.1 ml was injected intraperitoneally to the guineapigs.

Clinical study.—The extent of pulmonary lesion was assessed by radiograpic analysis and the patients were graded according to the classifications approved by the National Tuberculosis Association and the Trudeau Society of America. In majority of the patients the disease had lasted more than two years and every one had some sort of antitubercular therapy, though inadequately.

Since antitubercular therapy was given immediately the disease was diagnosed as tuberculosis or in many instances on mere suspicion, it was not

possible to obtain untreated pulmonary tuberculous patients. Neither the onset nor the duration of the disease prior to admission to the sanitoria could be definitely stated. Hence the investigations were limited to female tuberculous patients, majority in the age group 20 to 40 yrs, with fairly wide spread pulmonary lesions, sputum positive for tubercle bacilli and the disease process showing activity as gauged by fever, loss of weight and onset of other complications. Most of the patients were being given streptomycin and INH and a few were on PAS also. The clinical status of these patients is shown in Table V.

Whole blood clotting time(Lee and White, 1913).—Venous blood was drawn in dry sterilized syringe, time noted and 0.5 ml taken in clean, dry, glass test tubes. The tubes were tilted at intervals and time taken for complete coagulation of the blood observed, the end point being the complete invertion of the tube without flow of any blood.

Calcium clotting time (Biggs and Macfarlane, 1957).— 0.2 ml of citrated plasma was added to 0.2 ml of 0.85 per cent saline and the tube placed in the water bath at 37°C. 0.2 ml of M/40 calcium chloride was added and the time taken for the coagulation of plasma noted.

One stage prothrombin time (Quick, 1935).—0·2 ml of plasma and 0·2 ml of brain thromboplastin were mixed in a standard tube and placed in water both at 37°C; 0·2 ml of M/40 $CaCl_2$ was added and the time taken for a firm clot to form was noted.

Preparation of brain thromboplastin.—Fresh sheep brain was collected and all the superficial blood vessels and meninges were removed. The substance was macerated with 3 to 4 times its volume of acetone in a mortar. The acetone extraction was carried out 3 to 4 times and the granular powder dried in a suction filter. 0·5 g of this dried material was suspended in 10 ml of 0·85 per cent saline and the mixture was incubated at 37° for 1½ hrs. The suspension was mixed once or twice during this period and the coarse particles allowed to settle down by gravity. The concentrated solution was then standardised against fresh bovine plasma, so as to give the clotting time as 13. The dilutions of the thromboplastin were made with saline.

The two stage prothrombin (Dreskin, 1958).—The method is based on the use of a natural thromboplastin derived from hemolyzed red blood cells. A high yield of prothrombin is obtained and measured by a relatively simple method.

9.8 ml of aqueous calcium chloride (0.02 M) was added to 0.2 ml of citrated or oxalated blood and then incubated at 37°C. At intervals of 6, 10 and 14 min, 0·2 ml of aliquot were added to 0·2 ml barium plasma (prepared by adding 1 ml of 30 per cent barium sulphate suspension in water to 5 ml of pooled fresh oxalated plasma and incubating it for 10 mins at 37° with occasional mixing. After centrifugation and decantation, the prothrombin free plasma was obtained).

Calcium chloride was buffered to pH 7·3 to 7·4 by mixing $CaCl_2$ 2·22 g, 0·1 M barbital sodium 60 ml, 0·1N HCl 40 ml and water sufficient to make 1000 ml.

Fibrinogen estimation (Setna and Attman, 1956).—1 ml of plasma was mixed with 25 ml of saline 0·85 per cent and 1 ml of 2·5 per cent calcium chloride solution was added to it. After 1 hr at room temperature the fibrin clot formed was filtered off, washed three times with 0·85 per cent saline, transferred to a digestion flask and digested with 1 ml of concentrated H_2SO_4, till it was perfectly clear, which usually took about 4 hrs.

The nitrogen estimation was done by microkjeldahl method and the protein was calculated by multiplying it by 6·25 (the protein conversion factor).

RESULTS AND DISCUSSION

Expesimental tuberculosis of guinea pigs.—The quantitative analysis of the coagulation components in normal guineapigs is shown in Table 1. The data for the same animals during the progressive stage of tuberculosis together with the statistical analysis of the results is presented in Table II.

TABLE I

Coagulation components of blood in normal guineapigs

Serial No.	Clotting time			Prothrombin time		Fibrinogen
	Whole blood		Calcium	Stage one	Stage two	mg/100 ml plasma
	Min	Sec	Sec	Sec	Sec	
1	4	35	46	40	30	222.1
2	3	24	60	36	51	202.0
3	3	20	81	25	32	259.1
4	2	35	70	45	26.5	207.8
5	3	58	70	37	35	180.8
6	3	45	47	33	36	198.8
7	3	15	80	32	43	251
8	2	40	53	43	20	266.7
9	4	30	60	32	30	234.0
10	3.0	75.0	75	29	57	292.4
Mean±S.E.	3.30′±13.2″		64.2±4″	35.2″±2″	36.5″±3.6″	231.3±11.0

TABLE II

Coagulation components of blood in experimental gunieapig tuberculosis

Serial No.	Clotting time			Prothrombine time		Fibrinogen
	Whole blood		Calcium	Stage one	Stage two	mg/100 ml plasma
	Min	Sec	Sec	Sec	Sec	
1	3	14	40	56	56	601.3
2	3	3	54	42	55	465.2
3	1	37	42	49	60	530.2
4	2	44	44	47	72	506.7
5	2	14	33	51	50	362.9
6	2	45	52	49	52	252.1
7	3	5	42	41	61	54 0.3
8	2	45	43	50	60	525.9
9	3	5	40	46	70	478.5
10	2	20	51	45	53	614.4
Mean±S.E.	2′41″±32″		44.1″±6″	47.6″±1.4″	70.9″±2.3″	487.7±34.7
P	not significant		<0.01	<0.01	<0.01	<0.01

The final effect on coagulation seems to be one of accelarated clotting process. A definite decrease in the whole blood clotting time was obtained. The calcium clotting time also showed a statistically significant lessening.

The fibrionogen content was doubled from about 230 mg per cent to 487 mg per cent. The prothrombin times, as determined by both one stage and two stage methods were prolonged, more marked in the two stage, indicating a diminished prothrombin concentration in the blood. The fibrionolytic activity as described in our earlier paper was enhanced during infection.

Coagulation components in clinical tuberculosis.—The coagulation components in normal healthy women are shown in Table III. Table IV gives the findings in the tubercular patients. The clinical status of these patients may be seen in Table V. The trend of variations in the experimental and clinical tuberculosis is summarised in Table VI.

TABLE III

Coagulation components in healthy woman

Serial No.	Clotting time: Whole blood Min	Whole blood Sec	Calcium Min	Calcium Sec	Prothombin: Stage one Sec	Stage two Sec	Fibrinogen mg/100 ml plasma
1	6	46	3	37	66	24	232·7
2	4	40	3	37	51	15	231·3
3	3	50	3	31	50	15	167·3
4	5	30	3	38	54	22	152·4
5	4	35	3	39	46	29	160·8
6	4	35	3	11	51	18·5	195·9
7	3	35	2	25	54	16	277·5
8	3	50	2	26	59	18	186·4
9	3	55	2	48	54	19	242·2
10	4	15	3	31	60	21	197·2
11	3	0	2	45	70	19	184·9
12	5	30	3	35	68	19	216·0
13	4	40	4	4	73	19	162·8
Mean ± S.E.	4′	30″±16·4″	3′	17″±6·2″	58·2″±2·4″	18·9″±0·7″	200·5±10.4

TABLE IV

Coagulation components in clinical tuberculosis

Serial No.	Clotting time: Whole blood Min	Whole blood Sec	Calcium Min	Calcium Sec	Prothrombin time: Stage one Sec	Stage two Sec	Fibrinogen mg/100 ml plasma
1	3	0	2	7	68·5	28	227·2
2	7	40	4	32	91	21·5	140·2
3	5	10	3	40	75	21·5	503·4
4	9	15	4	53	62	20	348·2
5	4	0	3	23	71	23	412·2
6	4	10	3	52	61	24	428·5
7	5	5	4	30	77	23	159·2
8	5	10	3	0	73	27	253·0
9	5	0	3	58	85	16	389·1
10	6	15	5	18	76	16	382·9
11	5	30	2	45	65	27	221·8
12	5	15	3	42	68	23	342·9
13	4	45	2	23	68	30	542·9
14	7	0	3	4	75	24	264·0
15	7	40	3	36	73	20	267·2
16	7	40	5	33	79	20	261·9
17	5	40	3	59	74	16·8	5030
18	6	25	3	19	77	16	513·1
19	5	20	2	25	82	15	538·6
Mean ± S.E.	5′	47″±21″	3′	38″±1·7″	73·7″±1·7″	21·6″±1.0″	350·8±28·7
P					<0·1		<0·01

The serial numbers refer to patients shown in Table V

TABLE V

Clinical Status of tuberculosis patients

Serial No.	Age Yrs	Married	Children	Grade[1]	Fever	Sputum (Acid fast Bacilli)[2]	Remarks
1	24	Yes	2	II	—	+	Hemoptysis 1 month before
2	24	,,	3	III	+	+	...
3	24	,,	1	III	+	+	Blood stained sputum
4	38	,,	2	III	+	+	Diarhoea
5	20	,,	Nil	III	—	—	Laryngeal tuberculosis
6	30	,,	,,	III	Sl	+	...
7	24	,,	1	III	—	+	...
8	24	,,	4	III	—	+	...
9	40	,,	Nil	III	—	—	...
10	20	,,	1	III	—	+	...
11	20	,,	Nil	III to IV	—	++	Anasarca
12	26	,,	3	IV	+	+	Diarrhoea, oedema
13	21	,,	Nil	IV	+	+	Haemoptysis, coagulants given
14	25	,,	3	IV	—	+	Cervical lymphadenitis
15	43	,,	1	IV	+	+	...
16	45	,,	Nil	IV	—	+	Diarrhoea
17	14	No	...	IV	—	+	..
18	22	Yes	1	IV	—	+	...
19	27	,,	Nil	IV	+	+	Blood stained sputum

Fever — = no fever
Sl to + = various degrees

— = No acid fast bacilli
+ = Acid fast bacilli few
++ = Many acid fast bacilli

[1]Grade I. Slight infiltration without demonstrable cavitation.

II. Involvement of a small part of one or both lungs. Total volume of involvement regardless of distribution shall not exceed the equivalent of lung tissue which lies above the second chondrocostal junction and spine of the fourth thoracic vertebrae.

III. Increased infiltration with or without fibrosis, not exceeding more than the cavities, if present not exceeding 4 cms.

IV. Lesion more extensive than in III or definite evidence of greater cavity formation than III.

TABLE VI

Summary of the trend of variations in coagulation components in experimental and clinical tuberculosis

		Guineapig tuberculosis	Clinical tuberculosis
Clotting time	Whole blood	Decreased	Increased
	Calcium	Decreased	Increased
Prothrombin	Stage 1	Increased	Increased
	Stage 2	Increased	Increased
Fibrinogen		Increased	Increased
Fibrinolytic activity		Increased	No increase

The whole blood and plasma clotting times are increased but the trend of changes in the other coagulation components are similar to those of guinea pig tuberculosis. The prothrombin times as measured by both one stage and two stage techniques are prolonged and the fibrinogen content increased. No enhancement of fibrinolytic activity was observed (Chiplunkar and Sirsi, 1962).

The whole blood clotting time and the calcium clotting time reflect the time required for the reactions occurring during the lag phase and measures the efficacy of the early phase of clotting mechanism involving blood thromboplastin formation. Platelet factor and thromboplastinogens found freely in the plasma are the components likely to be involved in the variations of the whole blood clotting time. The role of these factors in acceleration of the clotting in acute guineapig tuberculosis and prolongation in clinical cases needs elucidation.

One stage prothrombin time.—The interpretations of the one stage method is rather difficult. The clotting time by the one stage method presents the resultant effects of the amount of thrombin formed, the speed of its formation and the reactivity of the fibrinogen and measures the summation of prothrombin and accellerator globulin rather than prothrombin alone. Though the test does not give a reliable measure of prothrombin, still as a practical guide to indicate the probable tendency of the patient to bleed, the results are found useful. In both guineapig tuberculosis and in the tuberculous patients significant decrease in the prothrombin concentration was observed.

Two stage prothrombin time.—The method adopted here is based on the use of hemolysed whole blood thromboplastin. The two stage prothrombin

method measures only prothrombin and is more specific and accurate than the one stage method.

The two stage prothrombin time of guineapig tuberculosis shows a statistically significant increase. In clinical cases too the same tendency was seen though the results were not highly significant.

The increased prothrombin seen in the first stage is due to decreased prothrombin content and not to other accelarator factors is clearly evidenced by the results of the two stage method.

The prothrombin content of the blood is generally known to be decreased in liver dysfunction and vit. K deficiency. In acute tuberculosis of guineapigs liver is studded with tubercular lesions and this may be one of the factors for prothrombin reduction. The interference with liver function is already reflected in the change of the albumin/globlulin ratio as shown in our earlier studies (Indira and Sirsi, 1959).

An interesting observation in this connection is that in guineapig tuberculosis, inspite of prothrombin deficiency, the whole blood and calcium clotting times were decreased and the blood clotted earlier. This might be due to increased availability of thromboplastic material as a result of tissue destruction and the elevated fibrinogen content which to a certain extent might neutralize the expected result of prothrombin deficiency.

In contrast to the above findings, in chronic tuberculous patients, associated with prothrombin deficiency, the whole blood and calcium clotting times were prolonged inspite of fibrinogen elevation.

The specific or multiple factors involved in the manifestation of accelaration of blood coagulation or prolonged clotting time are not well defined. A noxious stimulus or an acute stress can cause a prolongation in clotting time (De Long *et al.*, 1959), while excitement (Jaques, 1954), intravenous injection of sodium oxalate or intramuscular administration of sodium citrate (Richardson *et al.*, 1961), shortens the clotting time. Variations in dosages also can cause differential reactions. Blood coagulation is hastened by small doses and delayed by large doses of colloidal dyes (Sollmann, 1953). These examples are quoted to indicate how widely divergent are the etiological factors which do result in a common end effect.

Fibrinogen.—As regards the fibrinogen content, this is elevated in both the guineapig and clinical tuberculosis, being more prominent in the former.

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Fibrinogen.—As regards the fibrinogen content, this is elevated in both the guineapig and clinical tuberculosis, being more prominent in the former.

Fibrinogen concentration is also known to be affected by diverse factors: tissue damage from any cause whatsoever, raises the fibrinogen level. Bacterial diseases, virus diseases, trauma and cancer generally produce an elevation of the fibrinogen level (Wycoff *et al.*, 1959). The basic cause of this phenomenon has not been extensively investigated but most observers agree that dead or non specific (aseptic) inflammation also can cause elevation of fibrinogen (Foster and Whipple, 1921 ; Mann and Curtis, 1932). This elevation of plasma fibrinogen seems to be a common response to disease or injury. The endogenous polysaccharides released by damaged tissues are incriminated as causative factors for this pathological change (Laundy and Shear, 1957). In tuberculosis, tissue damage, disintegration of the mycobacteria, release of polysaccharides of both tissue and bacterial origin and hypersensitivity inflamatory reactions are all operating and the role of individual factors has to be assessed. Since there seems to be a correlation between the extent and rapidity of damage and the fibrinogen content, as seen by the higher ratio in the acute process of guineapig tuberculosis as compared to the chronic clinical disease, whether the estimation of fibrinogen would be helpful in predicting the progress of the disease remains to be examined.

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