CLINICAL TRIALS OF A.C.T.H. IN HAEMOLYTIC ANAEMIA

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Gardner (1950) has reported three cases of acquired haemolytic anaemia treated with 60–100 mg. of A.C.T.H. daily. One case showed a decrease in the osmotic and mechanical fragility of the red blood cells to normal, with a fall in the Coombs titre. Transfusions became unnecessary. Similar but less marked improvement was noted in the other two patients. Two of the patients responded to a second course of A.C.T.H. Gardner concluded that the hormone seemed to be temporarily effective in suppressing agglutinins and haemolysins active against the patient's own cells.

Dameshek (1950) refers to two patients with acquired haemolytic anaemia associated with generalized lymphosarcoma who showed striking improvement on A.C.T.H., with a fall in bilirubin and the antibody content of the serum, and an improvement of the lymphosarcomatous process. Relapse followed cessation of treatment, but improvement occurred with the institution of maintenance therapy. He also describes two other patients with acquired haemolytic anaemia who failed to respond to splenectomy but improved during the administration of A.C.T.H. In all four cases no further blood transfusions were required, although previously many transfusions had been necessary to maintain the blood counts at reasonable levels.

It is the purpose of this communication to report the results of the administration of A.C.T.H. in three cases of haemolytic anaemia.

Case 1

The patient, a married woman aged 30, had no family history of jaundice. Her parents, four brothers, and two sisters are alive and well. Her only child is healthy. She had never been seriously ill until 1948, when she developed "pneumonia." Penicillin and sulphonamides were given, and she was confined to bed at home for approximately three months on account of weakness, palpitations, and dyspnoea on effort. About this time she first complained of pain and swelling of the wrists and hands. She was referred to hospital as an out-patient, and a diagnosis of rheumatoid arthritis was made. The highest recorded blood sedimentation rate at this time was 87 mm. in one hour, but no records of the haemoglobin level or red cell count are available. The Wassermann reaction was negative. She received weekly injections of polyvalent vaccine throughout the following year. Her hands and wrists continued to trouble her, but, apart from occasional stiffness of the knees, no other joints were affected. She received two injections of gold, but the drug was stopped on account of albuminuria. Breathlessness and generalized weakness persisted.

She first came under our care when she was referred to the rheumatic clinic on May 28, 1950. Examination then revealed soft swellings on the dorsum of both wrists and hands, but the appearances were not typical of rheumatoid arthritis, and radiological examination was negative. The patient was pale and distressed. The haemoglobin level was 59% (Haldane). She was admitted to hospital nine days later. In the interval her condition had deteriorated. Breathlessness and pallor were much more pronounced, and jaundice had now appeared.

Examination revealed impairment of air entry at the left base. Radiological examination showed inflammatory changes at the left base consistent with a resolving pneumonia. The spleen could not be palpated, but there was tenderness in the left subcostal region. The liver was not enlarged, and there was no lymphadenopathy. The results of the laboratory and haematological investigations are shown in the accompanying Chart. Peripheral blood films revealed poorly filled red cells, anisocytosis, poikilocytosis, and occasional normoblasts. No abnormal white cells were noted. Sternal puncture showed a very active normoblastic marrow. Partial haemolysis of red cells occurred in 0.6% saline, and was complete in 0.5% saline. The direct van den Bergh reaction was negative, the icteric index 15 units, and serum bilirubin 1.6 mg. per 100 ml. The patient's

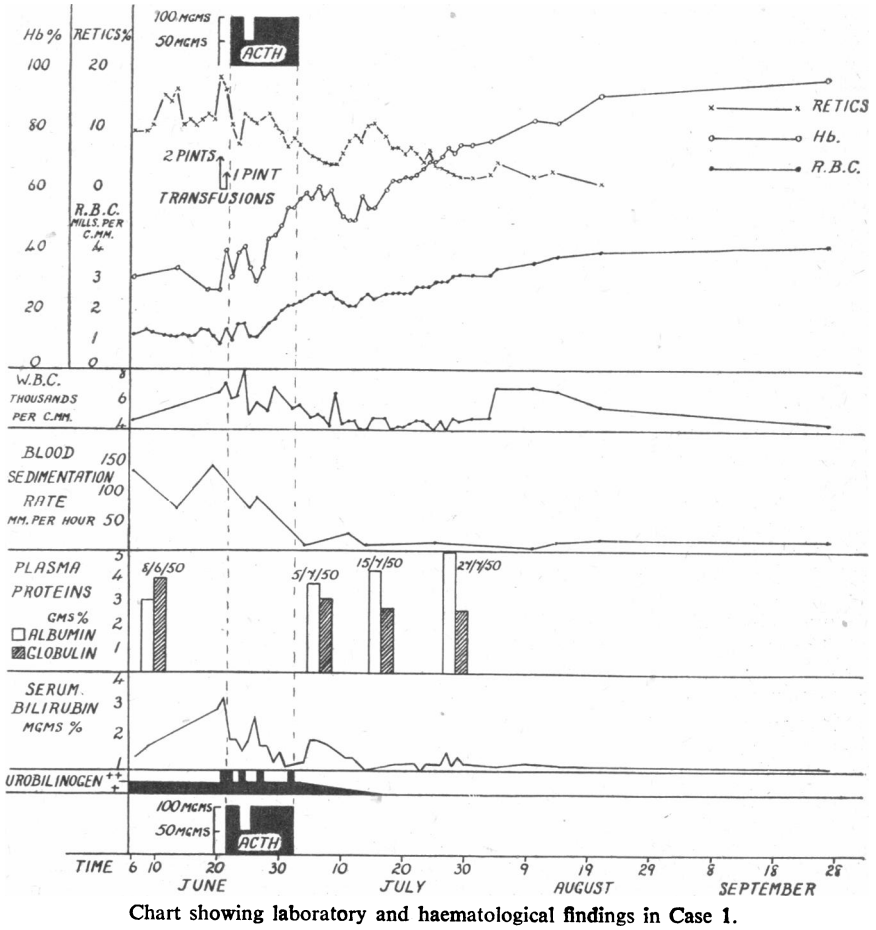

Chart showing laboratory and haematological findings in Case 1.

red cells were strongly agglutinated when suspended in her own serum, in normal serum, and in normal saline. Agglutination remained stable up to a temperature of 50° C. At this point it was dispersed and did not reappear on cooling. After agglutination of the patient's cells in saline had been dispersed by heat, the suspension was stored overnight in the ice-chest. The degree of haemolysis which had taken place at the end of 24 hours was compared with that in a suspension of normal cells in saline which had been heated and stored in the same way. Haemolysis of the patient's cells was almost complete, whereas in the preparation of control cells haemolysis was minimal. Washing the patient's cells did not materially alter agglutination in saline or the degree of haemolysis in the test described. The results of the direct anti-human globulin tests (Coombs) were difficult to interpret because of the agglutination and subsequent haemolysis. The Kahn test was strongly positive. The patient's clinical condition deteriorated as indicated by increasing dyspnoea, rising pulse rate, and the appearance of peripheral oedema. The signs of active haemolysis became more evident; reticulocytes rose, and there was a further fall in the haemoglobin level. Accordingly a total of 3 pints (1.7 litres) of packed cells was given. The beneficial effects were very transient.

On June 22, 1950, A.C.T.H. was administered in doses of 100 mg. daily (25 mg. four times a day). The patient was put on a low-salt diet in view of the possibility of salt and water retention. There was a dramatic improvement in her general condition within a few hours. Tenderness in the splenic region disappeared, dyspnoea became less troublesome, joint pain and swelling diminished, and appetite improved. On the morning of the third day the haemoglobin level had risen from 32% to 40% and the serum bilirubin had fallen from 3.2 mg. to 1.8 mg. per 100 ml. At this point the daily dose of A.C.T.H. was reduced to 50 mg. There was a prompt fall in haemoglobin level and a rise in reticulocytes and serum bilirubin (see Chart). The direct eosinophil count rose, indicating that the dose of A.C.T.H. was no longer sufficient to produce maximum adrenal response.

An increase of the daily dose to 100 mg. again produced a marked improvement in the patient's condition. This dose was continued for seven days. A few days after A.C.T.H. was stopped signs of haemolysis returned, as indicated by a rise in reticulocytes and serum bilirubin, with a fall in the red cell count. These signs quickly disappeared without further treatment, and progress thereafter was uninterrupted. Radiological examination showed that the inflammatory changes previously present at the left base had disappeared. No evidence of relapse had appeared when the patient was last seen on January 24, 1951.

During the period of improvement which followed the course of A.C.T.H. (July 25 to September 28) the following changes were noted. (1) The plasma proteins, which were reversed before the administration of A.C.T.H. (albumin 3 g. %, globulin 3.75 g. %), returned to a normal pattern (see Chart). (2) Agglutination of the patient's cells by her own serum persisted, but it became possible to prepare stable suspensions of her cells in normal serum and normal saline. After agglutination had been dispersed by heating to 50° C. the degree of haemolysis present, after 24 hours in the ice-chest, was comparable to that present in a suspension of normal cells in normal serum treated in the same manner. (3) The direct Coombs test became clearly positive, and the indirect test weakly positive. A weak non-specific cold agglutinin could be demonstrated.

The patient was discharged 54 days after admission completely free from symptoms. She was still well one month later and there had been a further improvement in the blood picture. Coombs's test was still positive, but there was minimal evidence of abnormal antibodies.

The patient was last seen on January 24, when she was in excellent health. Both the Coombs and Kahn tests were negative and all trace of abnormal antibodies had disappeared. The blood figures were: haemoglobin, 83%; R.B.C., 4,200,000; reticulocytes, 2%; serum bilirubin, 0.5 mg. per 100 ml.; E.S.R., 20 mm.

Case 2

The patient was a schoolboy aged 15. At the age of 5 years he had been admitted to the Royal Hospital for Sick Children, Edinburgh, with severe acute haemolytic anaemia, diagnosed as Lederer's anaemia. This responded to blood transfusions, and thereafter he remained well for eight years, until January, 1949, when, at the age of 13, he was admitted to the Royal Infirmary of Edinburgh with pallor, dizziness, vomiting, and night sweats of some two weeks' duration. At this time the haemoglobin was 52%, red cells 2,300,000 per c.mm., white cells 3,500 per c.mm., reticulocytes 6%, and there was a slight increase of the fragility of the red cells to hypotonic saline. A diagnosis of haemolytic anaemia was made.

From his history of previous illnesses it emerged that he had had operations for congenital hypertrophic pyloric stenosis at the age of 5 weeks, and for tonsillectomy and the removal of a tuberculous gland from the left side of the neck at the age of 2 years.

He was first seen by us in April, 1949, when he attended the blood clinic at the Royal Infirmary of Edinburgh. He now complained of dyspnoea on exertion, and his haemoglobin level varied between 74% and 91%. He was admitted to Professor Davidson's ward of the Royal Infirmary in October, 1949. There was no clinical icterus at this time, and the serum bilirubin was 1.3 mg. per 100 ml. The liver was not palpable, and the spleen was enlarged only to percussion. There was a slight increase in the fragility of the red cells to hypotonic saline, haemolysis beginning in 0.5% sodium chloride. The urinary urobilinogen was increased to 14.5 mg. a day and the faecal urobilinogen to 666 mg. daily. The bone marrow was normoblastic, and microspherocytes were present in the peripheral blood. Reticulocyte counts varied from 5% to 7%. The Wassermann reaction was negative.

The boy's parents and his brother gave no history of jaundice or of anaemia, and blood examinations revealed no abnormality. The paternal grandmother, who died in 1947, was said to have suffered from repeated attacks of jaundice.

Despite the lack of a definite family history, a diagnosis of congenital acholuric jaundice was made because of the presence of increased red-cell fragility and microspherocytosis, and the history of haemolytic crises in childhood. Accordingly splenectomy was advised, and the operation was successfully performed by Sir James Learmonth in January, 1950. The spleen weighed 290 g., and histological examination revealed evidence of moderately excessive blood destruction.

Unfortunately, however, no haematological improvement ensued, and the patient was readmitted to Professor Davidson's ward in September, 1950, with a haemoglobin level of 68%, erythrocytes 2,800,000 per c.mm., reticulocytes 4.8%. The marrow was normoblastic, and a differential count of the cells in the peripheral blood showed neutrophils to be 70%, lymphocytes 10%, and monocytes 20%. He continued to be dyspnoeic on exertion, and slight icterus was present. The liver was not palpable, but discrete glandular enlargement was found in the neck, groins, and axillae. Although the enlargement was only slight a gland was removed from the left side of the neck. The pathologist reported that there was reticulo-endothelial hypertrophy in the follicles, and infiltration of the sinuses with endothelial and round cells. The finding was compatible with chronic lymphadenitis or possibly a reticulo-endothelial hypertrophy following splenectomy. The Paul–Bunnell reaction and Kahn tests were negative, as was the direct Coombs test. There was no evidence of "cold" or other abnormal agglutinins as judged by the incubation of the patient's serum with standard cells suspended in saline and in 20% albumin at 4° C., room temperature, and 37° C.

TABLE I.—*Haematological Observations in Case 2, Treated with A.C.T.H.*

Date	Hb (%)	R.B.C. (Mill./c.mm.)	W.B.C. (per c.mm.)	Retics. (%)	Eosinophils. Lowest Daily Count (per c.mm.)	Red Cell Osmotic Fragility: Strength of Saline in which Haemolysis Began	Serum Bilirubin mg./100 ml.	Treatment with A.C.T.H.
26/10/50	73	3·14	10,800	7·4				
27/10/50				7·1				
28/10/50				6·8				
30/10/50	74	3·09	8,800	5·9				
31/10/50				5·0		0·48%	2·3	
1/11/50				5·5	413		2·1	
2/11/50	73	3·17	7,800	5·8	337	0·5%	2·1	
3/11/50				7·4	293	0·5%		
6/11/50	76	3·12	7,800	6·4	144		1·0	40 mg. b.d.
7/11/50			9,800	6·6	150	0·5%	1·0	40 ,, ,,
8/11/50	71		10,800	5·1	163		1·2	40 ,, ,,
9/11/50	74	2·95	13,200	6·8	25	0·5%	1·7	40 ,, ,,
10/11/50				7·6	19			40 ,, ,,
11/11/50	73		13,400	6·1				40 ,, ,,
13/11/50	76			8·2			1·7	
14/11/50				7·4		0·5%	1·5	
15/11/50	80	3·65	9,600	6·2	281		1·5	
23/11/50	78	3·46	8,600	5·8		0·5%		
11/1/51	73	2·82	14,800	4·7			2·3	

It was decided to treat the patient with A.C.T.H., and 40 mg. was given twice daily for six days. The various findings before and after treatment are given in Table I.

The effect of A.C.T.H. on the blood picture may be summarized as follows. It caused a marked fall in the eosinophil count, a rise in the total white cell count, and a moderate increase in the red cell count. Within a few days of the cessation of A.C.T.H. administration, all these findings had returned to the pre-treatment levels. The effect of A.C.T.H. on the haemolytic process was negative as judged by the failure to reduce the osmotic fragility of the patient's red cells to normal or to decrease the persistent reticulocytosis. The serum bilirubin fell from 2.1 to 1.0 mg. per 100 ml. on the first two days of treatment, but later rose to 1.7.

Case 3

The patient, a married woman aged 22, was known to have had anaemia in childhood and to have had four attacks of jaundice between the ages of 4 and 20 years. No evidence of a family history of haemolytic anaemia was obtained.

TABLE II.—*Haematological Observations in Case 3, Treated with A.C.T.H.*

Date	Hb (%)	R.B.C. (Mill./c.mm.)	W.B.C. (per c.mm.)	Retics. (%)	Eosinophils. Lowest Daily Count (per c.mm.)	Red Cell Osmotic Fragility: Strength of Saline in which Haemolysis Began	Serum Bilirubin mg./100 ml.	Treatment
7/12/50	80	3·81	8,200	5·8	150	0·6	3·2	
8/12/50				8·2	188	0·6	3·5	
9/12/50				9·4			3·1	
11/12/50	81	4·15	13,600	8·5		0·6		
12/12/50				8·1			3·4	
13/12/50				7·0			3·4	
14/12/50	81	4·10	12,400	11·2	175	0·6	3·8	25 mg. A.C.T.H. 6-hourly
15/12/50			8,600	7·6	125	0·6		25 ,, ,,
16/12/50			10,000	8·8	112	0·7	2·5	25 ,, ,,
17/12/50			9,900	7·2	92	0·7		25 ,, ,,
18/12/50	89		16,800	12·4	31	0·75	2·4	25 ,, ,,
19/12/50			13,600	13·6	87	0·75	2·9	25 ,, ,,
20/12/50	84	4·04	8,800	10·2	37	0·75	2·2	
21/12/50	88			9·8		0·75		
22/12/50	87	4·19		7·6	137	0·75	3·5	
23/12/50							2·9	
24/12/50						0·7	3·4	
25/12/50							3·1	
26/12/50	80	3·90	11,600	7·1	143	0·7		
27/12/50	85	4·12		6·8		0·7		
5/2/51	82	4·35	12,500	11·5				
6/2/51								Splenectomy
15/2/51	84	4·30	9,400	1·2			1·0	

On November 3, 1949, she was admitted to hospital on account of anaemia in the fifth month of pregnancy. Investigations at this time showed that she was suffering from haemolytic anaemia, the haemoglobin level being 75% and the reticulocyte count varying daily from 7% to 10%.

After giving birth to a healthy female child on April 8, 1950, the patient continued to suffer from haemolytic anaemia, and accordingly on December 6 she was re-admitted to hospital in order that A.C.T.H. therapy might be tried.

The patient felt well, but had slight clinical icterus. The spleen was enlarged 4½ in. (11.4 cm.) below the costal margin and the urine contained much urobilinogen. The marrow was hyperplastic and normoblastic. The blood figures were: haemoglobin, 80%; red cells, 3,810,000 per c.mm.; haematocrit, 29.5%; mean corpuscular volume, 77 μ^3; white cells, 8,200 per c.mm.; reticulocytes, 5.8%. Microspherocytes were present, and haemolysis began in 0.6% saline. Free acid was present in the gastric juice, and there was *x*-ray evidence of the presence of a gall-stone. The blood was Rh-positive, and, as on previous admissions, the direct Coombs test was negative. There was no evidence of "cold" or other abnormal agglutinins. The plasma proteins were normal and the Kahn test was negative.

It was decided to treat the patient with A.C.T.H., and 25 mg. was given six-hourly for six days, to a total dosage of 600 mg. The various findings before and after treatment are given in Table II.

The spleen became 2 in. (5 cm.) smaller at its tip within 24 hours of the beginning of treatment, and at the end of therapy it had become in all 3½ in. (8.9 cm.) smaller.

The effect of A.C.T.H. on the blood picture may be summarized as follows. There was no change in the haemoglobin or erythrocyte level. There was a fall in the eosinophil count and a rise in the total white cell count. The persistent reticulocytosis was not reduced nor was the osmotic fragility of the erythrocytes decreased, but there appeared to be a significant reduction of the serum bilirubin level from an average of 3.4 to 2.5 mg. per 100 ml. Within a few days of the cessation of treatment all figures had returned to the pre-treatment level. Accordingly, six weeks later splenectomy was successfully undertaken by Sir James Learmonth, and within a few days there was a fall in the reticulocyte count and serum bilirubin level.

Discussion

All the evidence in the first case would support the diagnosis of acquired haemolytic anaemia: the negative family history, the positive Coombs test, the transient effect of blood transfusions, and the presence of abnormal antibodies in the serum. With regard to the last-named, it would appear that two factors were involved: a powerful auto-agglutinin and an abnormal haemolysin. A somewhat similar case has been described by Dacie (1949). The administration of A.C.T.H. rapidly controlled the haemolytic process in Case 1.

A diagnosis of congenital haemolytic anaemia was made in Cases 2 and 3, despite the absence of a family history, because of the clear evidence of haemolytic episodes in childhood, the presence of increased fragility of the erythrocytes in hypotonic saline, the absence of circulating agglutinins or haemolysins, and negative Coombs tests. That the preparation of A.C.T.H. used was potent was indicated by the prompt fall in the eosinophil count, the rise in total white cell count, and the diminution in the size of the spleen in Case 3.

In contrast to Case 1 the only evidence in Cases 2 and 3 that the haemolytic process was temporarily affected was a moderate but significant fall in the serum bilirubin level in both cases. Whether larger doses of

A.C.T.H. or a longer course would have achieved any more striking results can be determined only by further clinical trials. All that can be said at the time of writing is that the effects of A.C.T.H. in the two cases of presumed congenital haemolytic anaemia were largely negative, in contrast to its dramatic effect in one case of acquired haemolytic anaemia.

In discussing the rationale for the use of A.C.T.H. in acquired haemolytic anaemia, Dameshek (1950) expresses the opinion that the beneficial effects may be due to a depression of antibody formation by the lymphoid tissue. Thorn (1950), on the other hand, feels that antibody formation may not be affected, but the reaction of the tissues to the antigen may be significantly altered. The fact that haemolysis was rapidly controlled in the first case while abnormal antibodies were still present does not support the view that the action of the adrenocortical steroids depends on a depression of antibody formation. An alternative hypothesis is that the damaging action of some product of the union of antigen and antibody is suppressed.

Summary

The results of the administration of A.C.T.H. in three cases of haemolytic anaemia are reported.

The administration of A.C.T.H. in a dose of 100 mg. daily for 10 days rapidly controlled the haemolytic phenomena in a case of acquired haemolytic anaemia, and symptoms have not recurred since.

In two cases of congenital haemolytic anaemia there was little or no response to the administration of A.C.T.H. in similar doses.

Evidence is brought forward to suggest that the control of haemolysis by A.C.T.H. in acquired haemolytic anaemia is not due to a suppression of the production of abnormal antibodies.

The A.C.T.H. used in these cases was supplied by the Medical Research Council, to whom we are indebted. We wish to express our gratitude to Dr. R. A. Cummings, Director of the Blood Transfusion Service, South-East Region, Scotland, who was responsible for the serological investigations.

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Speaking at Cardiff on March 12, the Minister of Health said the number of notifications of tuberculosis, which had been going up and down in the past 10 years, was now definitely showing signs of falling, and the number of deaths was going down. These developments were due to the applications of medical knowledge and skill supported by our National Health Service. In the years 1916–20 the average annual number of deaths from tuberculosis in England and Wales per million of the population had been 1,440; in 1940 it had been 670; in 1949 it had fallen to 450. Since the start of the National Health Service 3,550 additional hospital beds had been made available in England and Wales for the treatment of tuberculosis. He hoped that by the end of this year at least 2,000 more would be provided. Tuberculosis had for a long time been a more deadly enemy in Wales than in England. Mr. Clement Davies had pointed out in his report that, whereas in 1916–20 the mortality from tuberculosis had been the same in Wales as in England—namely, 1,440 per million of population—by 1936 the English figure had fallen to 680 and the Welsh only to 860. In 1949 the English figure was 415 and the Welsh 608.

EFFECT OF A.C.T.H. AND SUPRARENAL EXTRACT ON THE BONE MARROW*

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A connexion between the lymphoid tissues and the suprarenal cortex has been postulated with varying degrees of emphasis ever since Addison's (1855) classical monograph first appeared. Once Addison's disease had become recognized as a clinical entity, with its usually fatal termination, pathologists began to accumulate information about the lymphoid tissue hypertrophy which came to be regarded as one of the characteristic features of the disease, and which was even described as a "status lymphaticus" (Hedinger, 1907).

With the beginning of the modern study of the suprarenal cortex it became possible to investigate the action of individual hormones; and, of the two main groups of steroid hormones which the suprarenal cortex was known to produce, it soon became generally held that it was those with an oxygen atom at C 11 which were more especially associated with the lymphoid tissue changes. This view was challenged by Selye and his co-workers (Dontigny, 1946), but the demonstration (Hechter *et al.*, 1949), by perfusion of the isolated suprarenal with deoxycortone, that this substance could thereby be converted into 11-oxy compounds seemed to provide an obvious way of reconciling these conflicting points of view.

The most extensive work on the relation between the suprarenal cortex and lymphoid tissue is that of Dougherty and White (1945, 1947), who from 1943 to 1947 published a number of papers on the subject, and in 1947 summarized the results both of their own work and that of other investigators. Broadly speaking, their work followed two main lines. After the administration of adrenocorticotropic hormone and cortical hormones, they not only observed regressive changes in the lymphocytes and lymphoid tissue, but also attempted to correlate these with increased formation of gamma globulin and antibodies. The problem has been extensively reviewed elsewhere (Yoffey, 1950), and so far as antibody formation is concerned all that need be said here is that considerable doubt has been cast on either the increased production of antibodies in response to cortical hormones (Eisen *et al.*, 1947; Thatcher *et al.*, 1948) or the relationship between antibodies and lymphocytes (Fagraeus, 1948; Ehrlich *et al.*, 1949). It is doubtful, in fact, whether there is any correlation whatever between the lymphoid tissues and any of the plasma proteins (Andreasen *et al.*, 1948).

*Read by Professor Yoffey to the Section of Anatomy and Physiology at the Annual Meeting of the British Medical Association, Liverpool, 1950.