TABLE XI.—Time Convalescent in Weeks in Patients Considered by Age, Expressed in Actual Number Seen in Each Group (Excluding Patients Retired and Children)

Age	Weeks												Returned at 6 weeks
	0-2		3-4		5-6		7-12		13-18		18 +		
	N	%	N	%	N	%	N	%	N	%	N	%	
10-19 (12)	—	—	7	58.3	—	—	1	8.3	1	8.3	3	24.9	58.3%
20-29 (52)	6	11.5	17	32.7	13	25.0	12	23.0	2	3.8	2	3.8	69%
30-39 (108)	11	10.2	38	35.2	30	27.7	19	17.6	3	2.8	7	6.5	73%
40-49 (198)	14	7.2	60	31.1	50	25.8	47	24.3	13	6.7	9	4.6	64%
50-59 (168)	7	4.2	47	28	42	25.1	58	34.5	7	4.2	7	4.2	57%
60-69 (79)	6	7.6	26	32.9	20	25.3	21	26.6	4	5.1	2	2.5	65.8%
70-79 (23)	5	21.5	9	39.1	3	13.0	5	21.5	—	—	1	4.3	73%

TABLE XII.—Time Convalescent in Weeks in Patients Seen—Excluding Patients Retired and Children at Time of Operation—According to Type of Hernia

Type of Hernia	Weeks							0-6
	0-2	3-4	5-6	7-12	13-18	18+		
Indirect inguinal	6	31.4	25	27.8	4	5.8	62.4	
Direct "	2.8	30	30	25.7	7.7	3.8	62.8	
Femoral "	18.1	35.1	22.4	18.1	4.2	2.1	75.6	
Incisional	13.0	34.8	17.4	30.4	—	4.4	65.2	
Umbilical	11.5	19.3	23.1	23.1	11.5	11.5	53.9	

TABLE XIII.—Reason Stated by Patients Interviewed for Return to Work, Excluding Patients Retired or Children

Year	Own Wish	Own Doctor's Instructions	Hospital Doctor's Instructions	Works Doctor's Instructions
1948	65	23.8	8	3.2
1949	76.5	23.5	—	—
1950	60	35.6	2.2	2.2
1951	71.2	17.3	7.7	3.8
1952	70.2	19.4	10.4	—
1953	64	18	6	12
1954	58	27.6	9.6	4.8
1955	66	23.8	10.4	5.8
1956	65.5	14.8	12.3	7.4
1957	60	26.3	9.5	4.2

Discussion

In any discussion on convalescence it is realized that there are innumerable factors which will play a part in deciding the time actually taken to convalesce by any one patient. Table II is an attempt to list and clarify some of these points, which also have an equal bearing on recurrence of a hernia.

Most of the preoperative, operative, and post-operative factors can be factually reported. It was noted in this series that in every year there was a great diversity in all these factors, which did not follow any set pattern. But among the miscellaneous factors listed, certainly the decision to return to work cannot be evaluated, especially when it is realized that the majority of patients make up their own minds, apparently uninfluenced by their medical advisers. This is a salutary lesson. It is presumed that the decision is made when the patient feels able and fit to resume his occupation. Economic factors are doubtless of importance.

The observed result is that the majority of patients will return to work within six weeks. In 8 out of the ten years and in all but umbilical herniae, the highest number had only six weeks' convalescence from the date of operation. This means that most patients take only four weeks' rest at home. This appeared to have no malign effect upon the recurrence rate.

The length of convalescence did not vary with age or type of hernia. However, there was a tendency, as one would expect, for those patients in the lowest social class and performing the heaviest work to take longer over their convalescence. The incidence of recurrence of herniae in those cases seen did not vary significantly from year to year.

Summary

A review of 762 herniae is presented.

The diversity of factors involved in the treatment of herniae is discussed. The convalescent period is shown to be directly determined by the patient in the majority of cases. Most patients favour a period of two to four weeks (four to six from date of operation) after various types of hernia repair. This period is unrelated to age, type of hernia, work, or social status, except in class V and the heaviest work, but does vary as expected with the occurrence of complications.

Our thanks are due to Mr. E. G. Muir, Mr. R. S. Lawrie, and Mr. J. M. Pullan, under whose care these patients were admitted. We are also grateful to Mr. R. S. Lawrie for advice and criticism during the preparation of this paper. This work was generously supported by a grant from the South-West Metropolitan Hospital Board.

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A CLINICAL STUDY OF SERUM ANTINUCLEAR FACTOR

BY

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Since the description of the L.E.-cell phenomenon by Hargraves *et al.* (1948) and the rheumatoid factor by Rose *et al.* (1948) and Waaler (1940), immunological abnormalities in the connective-tissue diseases have been extensively sought for and studied.

In sera from patients with systemic lupus erythematosus (S.L.E.) a number of abnormal globulin factors have now been described, including autoimmune haemolytic antibodies (Zoutendyk and Gear, 1950), antiplatelet and anticoagulation factors (Dameshek, 1958; Loeliger, 1959), antileucocyte agglutinations (Seligman, 1958), and anticytoplasmic complement-fixing antibodies (Mackay and Gajdusek, 1958; Asherson, 1959; Deicher *et al.*, 1960).

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After the localization of the L.E.-cell factor in the γ -globulin fraction of serum by Haserick *et al.* (1950), Miescher (1957) used the antiglobulin consumption test to demonstrate the affinity of this factor for cell nuclei, and since then a number of other techniques have been used to show serum reactions with nuclear material, including the fluorescent antibody technique (Friou *et al.*, 1957, 1958; Holborow *et al.*, 1957; Holman and Kunkel, 1957; Alexander and Duthie, 1958), complement fixation (Ceppellini *et al.*, 1957; Pearson *et al.*, 1958; Seligman, 1958), precipitation (Ceppellini *et al.*, 1957; Seligman, 1958), haemagglutination (Miescher and Strässle, 1957; Goodman and Bowser, 1958; Lee and Epstein, 1960), and latex precipitation (Christian *et al.*, 1958; Fessel, 1959).

This paper is concerned with the serum antinuclear factor, which we have previously reported (Holborow *et al.*, 1957), and presents a study of its distribution in systemic lupus erythematosus, rheumatoid arthritis, and some other conditions.

Materials and Methods

The demonstration of the antinuclear serum factor utilizes Coons's indirect technique, in which globulin uptake by tissue sections is demonstrated by treatment with anti-human-globulin serum conjugated with fluorescein isocyanate or isothiocyanate. Sections pretreated with serum containing antinuclear factor (A.N.F.) show bright overall apple-green fluorescence of the cell nuclei (Holborow *et al.*, 1957) when viewed microscopically by ultra-violet light, whereas control sections pretreated with normal serum show no fluorescent staining.

Preparations of Tissue Sections.—The selection of cell nuclei in a suitable form for the test is an important consideration. Preparations of nuclei which we have tested include smears of cell nuclei prepared from tissue homogenates, human leucocyte films, and monolayers of cells grown in tissue culture. None of these preparations was ideal owing to non-specific uptake of serum protein, which leads either to excessive background staining or sometimes to non-specific nuclear staining. Experience with tissue-culture monolayers indicated the need for some cell damage before the nuclei could take up the serum factor. Freezing and thawing gave suitable preparations, but mild trypsinization resulted in considerable non-specific protein uptake by the cells. The most satisfactory nuclear preparations for routine use were made from thyroid tissue because of the orderly and predictable arrangement of the cell nuclei, and this tissue was used for the present study. Human infant thyroid tissue was preferred to adult tissue, because the latter usually contains numerous auto-fluorescent granules in the epithelial cytoplasm, and much interstitial autofluorescent collagen, both of which interfere with observation of the sections. Human neonatal thyroid from very fresh post-mortem specimens was cut into small blocks and instantly frozen in a sealed container in solid carbon dioxide and alcohol mixture (approximately -70° C.), transferred to a freezing cabinet at -20° C., and stored without thawing. Sections were cut in a cryostat chamber on a microtome at $6\ \mu$ and mounted on slides and dried as described by Coons and Kaplan (1950). Each section was covered with a few drops of test serum and incubated in a moist chamber at 37° C. for 30 minutes. After incubation the serum was carefully washed off

in two changes of buffered saline (pH 7) for 20 minutes, fixed in 90% alcohol for five minutes, washed again and stained with a fluorescent conjugate prepared from anti-human-globulin serum. After 30 minutes the sections were again washed and mounted in buffered glycerin (pH 7).

Preparation of Conjugates.—The anti-human-globulin sera were prepared in rabbits by intravenous injection of rabbit red cells coated with human globulin (Milgrom *et al.*, 1956). Alternatively a horse anti-human whole serum obtained from the Pasteur Institute was used. The globulin fraction was precipitated from the antiserum by 50% saturation with ammonium sulphate, and conjugated according to the methods of Coons and Kaplan (1950) or of Marshall *et al.* (1958). To reduce non-specific staining conjugates were absorbed with rabbit-liver suspension. This is particularly important, as background fluorescence makes differentiation between stained nuclei and the stained background difficult. Anti-human-globulin-fluorescein conjugates vary in their ability to detect traces of serum protein remaining adherent to treated washed tissue sections, and this can sometimes lead to quite considerable background fluorescence in preparations treated with all normal sera. The staining in these cases may include "outlining" of the cell nuclei, which leads to difficulty in interpreting the A.N.F. test. The majority of tests reported here were carried out using a conjugate which did not show this effect. Tests using other conjugates were standardized where necessary by diluting sera 1 in 5 or 1 in 10 with saline. At this dilution standard normal sera gave minimal background fluorescence of the type described. The overall nuclear fluorescence acceptable as positive in the test is thus easily recognized.

Controls.—Each series of tests included a known positive and a known negative serum control. The specificity of the conjugate was controlled by inhibition of staining by unconjugated antiglobulin and the absence of similar staining by fluorescent conjugates with different specificities.

Ultra-violet Microscopy.—A Cook, Troughton, and Simms microscope unit (M2150) with dark-ground condenser (M1396) was used. The light source was a 250-watt mercury-vapour lamp with choke and switch-gear (M1684).

L.E.-cell Test.—This was the clot method of Zimmer and Hargraves (1952) when fresh blood was available, or the indirect technique of Schultz *et al.* (1955) when serum only was available.

Selection of Sera

Systemic Lupus Erythematosus.—We have tested sera from 58 cases of S.L.E. diagnosed according to the criteria shown in the Appendix. This group consisted of 14 patients from this unit (Professor Bywaters), 12 patients from the Middlesex Hospital (Dr. Doniach), and 32 patients from the University Hospital, Leyden (Dr. Hijmans). In all these cases a positive L.E.-cell phenomenon had been demonstrated on one or more occasions. Five other Taplow cases which fitted the diagnostic criteria, but with negative L.E.-cell tests, were also included in the study.

Rheumatoid Arthritis.—This group was selected using the American Rheumatism Association criteria for diagnosis, and was from cases seen at this unit and

TABLE I.—*Antinuclear Factor in Sera of Cases of Liver Disease*

	No. Tested	A.N.F.-positive
Post-infective cirrhosis ..	9	4 (1 L.E.-cell positive)
Portal cirrhosis ..	4	1
Juvenile ..	10	0
Alcoholic ..	4	0
Primary biliary cirrhosis ..	3	0
Other types of ..	9	0
	39	5

TABLE II.—*Distribution of Positive A.N.F. and L.E. Tests in Disease Groups Studied*

Diagnosis	A.N.F.			L.E. Cells		
	No. Tested	Positive		No. Tested	Positive	
		No.	%		No.	%
S.L.E. ..	63	62	98	63	58	92
Discoid lupus erythematosus ..	75	10	13	75	0	0
Rheumatoid arthritis ..	132	19	14	95	3	3
Still's disease ..	100	13	13	60	1	2
Thyroid ..	110	14	13	107	0	0
Liver ..	39	5	13	39	1	3
Rheumatic fever ..	56	0				
Pulmonary tuberculosis ..	65	2				
Scleroderma ..	8	0				
Dermatomyositis ..	4	2				
Paget's disease ..	4	1				
Normal sera ..	133	2				

Hammersmith Hospital, many of which were undergoing long-term follow-up (Dr. Dixon and Professor Bywaters).

Still's Disease.—The cases were seen at this unit (Ansell and Bywaters, 1959).

Discoid Lupus Erythematosus.—Sera came from L.E.-cell-negative patients without signs of systemic disease (Scott and Rees, 1959).

Liver Disease.—This group of sera came from patients in Hammersmith Hospital and from Hamburg (Dr. Dölle). These patients are classified in Table I.

Results

Table II shows the results of A.N.F. and L.E.-cell tests on the sera from the cases described above. All 58 cases of systemic lupus with positive L.E.-cell tests gave positive A.N.F. tests at first testing, and none became negative subsequently. Four of the five L.E.-cell-negative S.L.E. cases were positive for A.N.F. and one was negative despite repeated testing. This case was that of a 21-year-old woman presenting six years ago with typical butterfly rash, loss of weight, pyrexia, loss of hair, generalized lymphadenopathy, splenomegaly, arthralgia, and leucopenia. Three years ago the E.S.R. returned to normal, and on steroid therapy she apparently recovered. Repeated tests for L.E. cells throughout her illness and (during the last three years) for A.N.F. have been negative.

In the rheumatoid arthritis group of 132 cases, 19 gave a positive A.N.F. test. Two of these were L.E.-cell positive and 17 were negative, but a third L.E.-cell-positive case was encountered which was persistently negative for A.N.F. In the group with Still's disease approximately the same incidence (13%) of positives was found.

The liver-disease group included nine cases of post-infective cirrhosis, and four out of the five positive A.N.F. tests occurred in sera from cases with this diagnosis. One of these four also gave a positive L.E.-cell test, another had haemolytic anaemia, and three out of the four had arthropathies. Joint involvement was present in only two out of the 34 A.N.F.-negative cases.

Positive A.N.F. tests have also been found in a significant percentage of cases of thyroid disease,

including Hashimoto's thyroiditis and thyrotoxicosis. This group will form the subject of another communication (Hijmans, Doniach, Roitt, and Holborow, to be published).

Apart from these, we have found only five other cases positive for A.N.F. among the cases shown in Table II. These were two cases of clinical dermatomyositis from this unit, one case of Paget's disease with congestive failure, and two cases of pulmonary tuberculosis, one with penicillin hypersensitivity (in which the A.N.F. titre was low). Two other cases of drug sensitivity were negative. We also found two positives among 133 sera from apparently normal individuals. Both of these were weakly positive, with titres no higher than 1/10. It will be seen that in 63 other cases of pulmonary tuberculosis, and in 56 cases of rheumatic fever (active and quiescent), no positive tests were encountered. We have also tested 56 miscellaneous hospital cases, including chronic bronchitis, coronary disease, pneumonia, congestive cardiac failure, asthma, and iron-deficiency anaemia, none of which gave positive tests.

Serum Titrations.—Titration of A.N.F. by serial dilution in saline was carried out in 36 cases, testing for A.N.F. under standard conditions using the same tissue and conjugate. The sera chosen for this investigation were selected from the four main diagnostic categories in which the factor has so far been found. Table III

TABLE III.—*Titration of Antinuclear Factor*

	No. Tested	No. Positive at Stated Dilution		
		Neat—1/10	1/20—1/100	>1/100—1/300
S.L.E. ..	14		6	8
Rheumatoid arthritis ..	12	1	9	2
Liver disease ..	4		4	0
Thyroid ..	6*	1	5	0
Dermatomyositis ..	1		1	0
Pulmonary tuberculosis ..	2	2	0	0
Normal sera (positives) ..	2	2	0	0

*Drs. Doniach and Roitt's cases.

gives the distribution of the titres according to the diagnosis, and shows that with two exceptions only S.L.E. sera could be diluted over 1/100 and still retain the ability to give a positive A.N.F. test. The low-titre group included some S.L.E. cases, most of the rheumatoid arthritis cases, and all the cases of liver and thyroid disease. Only four sera, in addition to the two normal sera described above, had titres of less than 1/10—one case of rheumatoid arthritis, one case of thyroid disease, and the two cases of pulmonary tuberculosis.

Discussion

The L.E.-cell phenomenon, which occurs in S.L.E. and sometimes in other diseases, has been shown to involve a serum factor reacting with nuclear material (Miescher and Fauconnet, 1954; Hijmans and Schuit, 1958). It is therefore possible that the antinuclear factor which we have described above represents the same serum factor. Our work does not enable us to draw any final conclusions on this point, because in many of the cases studied only serum, not fresh blood, was available, and therefore it was often possible to carry out L.E.-cell tests only by an indirect method. Studies which have revealed a high incidence of positive L.E.-cell tests in rheumatoid arthritis, for example, have used a sensitive direct method for the L.E.-cell test (Kievits *et al.*, 1956). In our hands, nevertheless, A.N.F. has had a much wider incidence in the disease groups studied than positive direct L.E.-cell tests. Thus, of the 19 rheumatoid arthritis patients positive for A.N.F.,

only two gave positive L.E.-cell tests by the direct method. In this group, however, titration of the A.N.F. in L.E.-cell-negative cases, where it was carried out, showed a low titre. On the other hand, we have encountered sera with A.N.F. titres of 1/100 where direct L.E.-cell tests were negative. Occasionally we have failed to find A.N.F. in sera from cases giving positive L.E.-cell tests, as in the rheumatoid arthritis case described above. Marmont (1959), however, has suggested that, in rheumatoid arthritis especially, apparently typical L.E. cells may sometimes be found in the absence of a specific serum factor, and it is possible that this L.E.-cell-positive and A.N.F.-negative case may belong to this category.

The significance of A.N.F. in the disease groups studied may be considered under two headings—diagnosis and aetiology.

Our results show that A.N.F. was present in all our cases of clinically definite S.L.E., except one in prolonged remission, discussed above. The difficulty of diagnosing borderline cases (particularly in the absence of L.E. cells) makes it impossible to assert that A.N.F. is present from an early stage in every case of the disease. Our experience indicates that once the factor has been found in S.L.E. it is likely to be continuously present thereafter, and its high correlation with proved S.L.E. makes this diagnosis unlikely in its absence. A positive test (as already indicated) is not by itself diagnostic of S.L.E.

Because of its antibody-like nature A.N.F. may be classed with the autoimmune phenomena characteristic of systemic lupus. If we accept A.N.F. as an indicator of autoimmune processes, its occurrence in some cases of discoid lupus confirms the aetiological relationship between this and the systemic form of the disease. In the same way the incidence of A.N.F. which we have found in the adult and juvenile forms of rheumatoid arthritis, while it may not indicate the true incidence because of case-selection factors, nevertheless suggests that an immunological abnormality comparable to that found in S.L.E. may exist also in rheumatoid arthritis. The finding of A.N.F. in cases of liver disease accords well with the work of Mackay and Gajdusek (1958), showing that in certain forms of cirrhosis autoimmune complement-fixing antibodies (A.I.C.F.) and L.E. cells may be found. It is possible here, however, that disordered serum-protein synthesis rather than an initial autoimmune disturbance may give rise to these findings.

The role of these "auto-antibodies" in the pathogenesis of S.L.E. is doubtful. The fact that infants of lupus mothers do not develop the disease despite the presence in their circulation at birth of L.E.-cell factor (Bridge and Foley, 1954), and that an S.L.E.-like disease and associated conditions occur in agammaglobulinaemic individuals (Good, 1960), suggests that circulating antibody alone cannot be responsible for the pathology of the disease.

The abnormality of the immunological mechanisms in S.L.E. responsible for the wide range of auto-antibody γ -globulin found in this condition is not understood, but the lack of organ specificity (and indeed of species specificity) shown by many of the auto-antibodies (including A.N.F.) which occur in this disease appears to distinguish it broadly from autoimmune states such as thyroiditis, encephalomyelitis, and aspermatogenesis.

The production of these auto-antibody factors in S.L.E. can be regarded as a breakdown in immune

tolerance to the body's own tissue antigens. Thus Burnet (1959) envisages development of clones of antibody-forming cells capable of reacting with self, owing to a poorly functioning homeostatic mechanism failing to suppress such mutant cells. If such malfunction were marked it would be likely, as Burnet suggests, to lead to a multiplicity of antibodies accompanying the emergence of many "forbidden clones." In rheumatoid arthritis, however, one may postulate that the abnormality is less marked and that the homeostatic mechanism is less affected. In these circumstances only a limited number of forbidden clones emerge, and, so far as antinuclear factor is concerned, in only a proportion of cases. If we accept this view, it seems reasonable at present, on the basis of A.N.F. activity, to regard rheumatoid arthritis as a disease immunologically related to S.L.E.

Summary

Serum antinuclear factor (as shown by Coons's fluorescent antibody method) has been found in 62 out of 63 cases of systemic lupus erythematosus and in over 10% of cases of discoid lupus erythematosus, rheumatoid arthritis (including Still's disease), and liver disease. The nature of the factor is discussed in the light of current concepts of autoimmunity.

We are grateful to Professor E. G. L. Bywaters for help in the preparation of this paper, and for providing, with Dr. B. M. Ansell and Dr. Allan Dixon, many of the cases we have studied. Our thanks are also due to Dr. Deborah Doniach, of the Middlesex Hospital, and Dr. W. Hijmans, of the Rheumatology Department, University Hospital, Leyden, The Netherlands, for making available sera and clinical information from their cases of systemic lupus. Dr. Allene Scott and Dr. E. G. Rees, of St. Bartholomew's Hospital, kindly provided sera from their cases of discoid lupus, and Dr. W. Dölle from cases of liver disease.

APPENDIX. CRITERIA FOR SELECTION OF CASES OF LUPUS ERYTHEMATOSUS

Obligatory.—The sedimentation rate must be above 20 mm. in one hour, Westergren or Wintrobe.

Major Criteria.—(1) Rash compatible with disseminated lupus. (2) Leucopenia below 5,000. (3) Finding of L.E. cells in 15-minute search of one slide.

Minor Criteria.—(1) Arthralgia or effusions into joints. (2) Serositis. (3) Fever above 99° F. (37.2° C.) twice in 24 hours. (4) Retinal changes. (5) D.A.T. 1:16 or more. (6) Albuminuria above 5 mg./100 ml.

For the admission diagnosis the patient must have the obligatory criterion, plus two or more major criteria, or the obligatory criterion, plus one major and at least two minor criteria. These criteria were those submitted by Bywaters and Ansell to the M.R.C. Collagen Panel, 1952.

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PURE RED-CELL APLASIA IN MARASMUS AND KWASHIORKOR TREATED WITH RIBOFLAVINE*

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During an investigation of the anaemias in marasmus and kwashiorkor it was found that a considerable proportion of patients developed pure red-cell aplasia or hypoplasia during treatment.

So far selective red-cell aplasia has not been reported as a complication of marasmus and kwashiorkor, perhaps because critical haematological work and bone-marrow cytology have not been extensively done in these conditions, and serial bone-marrow punctures are not commonly performed on small infants. As the aplasia does not usually develop until two weeks or more after admission, a single marrow examination done at that stage, when red-cell precursors are usually high, will not reveal a hypoplasia that is yet to appear.

From the present investigation it seems that this type of aplasia is a fairly common accompaniment of

marasmus and kwashiorkor in Kenya, since it developed in nine instances during the treatment of 23 cases; of these, five were thoroughly investigated.

Red-cell aplasia probably has a number of different causes among which are thymomas (Chalmers and Boheimer, 1954; Jacobs *et al.*, 1959; Freeman, 1960), toxic agents, allergy, iso-immunization, and infections (Foy and Kondi, 1953).

The case of pure red-cell aplasia previously reported (Foy and Kondi, 1953) and successfully treated with riboflavine induced us to try this vitamin in the present cases, and the results are reported here.

Material and Methods

Typical cases of marasmus and kwashiorkor attending the King George VI Hospital, Nairobi, were diagnosed clinically by the criteria of Dean and Schwartz (1953), Scrimshaw *et al.* (1957), and Jelliffe and Dean (1959). On admission complete haematological and parasitological examinations were done, together with x-ray examination to discover any chest infection.

All cases were then admitted to the children's ward, where dietary treatment consisted of "casilan," skimmed milk, and vegetable oil mixture (Jelliffe and Dean, 1959). They were covered during the first week with either tetracycline or penicillin and streptomycin. Intercurrent infections, such as hookworm, giardiasis, malaria, measles, diarrhoea, and respiratory complaints, when present, were appropriately treated. As most of the patients had low haemoglobin (Hb) and mean corpuscular haemoglobin concentration (M.C.H.C.) levels, oral iron was given in appropriate doses.

Blood was taken from the internal jugular vein, and bone-marrow from the lumbar spinous processes. Blood and marrow examinations were done on admission and thereafter fortnightly, or more often, for from two to four months. Marrow iron was estimated by the direct unstained method (Rath and Finch, 1948; Wallerstein and Pollycove, 1958) and graded accordingly. For sideroblast estimations marrow smears were fixed with 10% formalin in absolute alcohol, washed in water, and the slide flooded with warm potassium ferrocyanide in 5N HCl, again washed in water and counterstained with 1% aqueous safranin. In searching for the red-cell precursors marrow particles were most carefully examined after staining with Leishman and Giemsa. Haemoglobin was determined by the cyanomethaemoglobin method, and the packed cell volume (P.C.V.) by the microhaematocrit. The proteins were estimated by the biuret method (Gornall *et al.*, 1949); fractionation was done by using 27.2% sodium sulphate, to avoid carrying over α_1 -globulin to the albumin and thereby giving falsely high albumin and low globulin values. Separation of the globulins was done by low-voltage paper electrophoresis at constant pH to reduce the albumin trail. Since there were great variations in the protein content of the serum, sometimes falling as low as 3 g./100 ml. of total protein, the amount of serum seeded on to the paper was calculated by the formula

$$\mu\text{l.} = \frac{35}{\text{T.P.}}$$

Case Histories: Treatment and Results

Case 1

A 12-months-old Kikuyu girl entered hospital on April 30, 1960, with typical marasmus/kwashiorkor and a three-months history of diarrhoea. Stools contained giardia; treated with mepacrine. Hb, 9.5 g./100 ml.; P.V.C., 33%;

*Riboflavine used in this work was kindly supplied by Roche Products Ltd.