beginning of the last century. The control of blindness in India is thus largely a problem of the control of infectious disease. That this is not purely an issue of pathology and pharmacology is obvious enough from the history of public health in Western Europe, and is grimly emphasized by the fact that keratomalacia—a nutritional deficiency disturbance—is ranked as first among the causes of blindness in children. The problem of blindness in India is not one of the elimination of a particular infectious disease such as trachoma or the acute ophthalmias. It has to be conceived in terms of standards of living, education, facilities for treatment, and adequate and well-trained ophthalmic personnel. None the less there is much that can be done immediately both outside and as part of the long-term plans—how much, can be seen more clearly from a consideration of the position in

Egypt and Palestine.—Here the social problems are not dissimilar in their broad aspects from those seen in India, but unlike in India two clearly defined infectious diseases are the main causes of blindness—the acute purulent ophthalmias and trachoma. Purulent ophthalmia, the more significant cause, is now easily treated by the sulphonamides and by penicillin. An immediate and striking reduction of blindness in Egypt, Palestine (and, indeed, throughout the whole of the Near and Middle East), and perhaps India, could be brought about by the organization of relatively simple treatment stations and possibly mobile units, with adequate supplies of the new chemotherapeutic and antibiotic agents. Trachoma, too, could be considerably reduced by such stations, for the beneficial effect of sulphonamide therapy in trachoma is no longer open to doubt; moreover, there are indications that penicillin may also be highly effective. The creation of such treatment stations will in any case have to be undertaken in any long-term programme, but a sense of urgency has now been supplied by the availability of the new therapeutic agents, for each day that such treatment is not available to the millions of Africa and Asia adds many blind to the roll of the avoidably blinded.

Summary

1. The number of blind in the British Isles is not less than 90,000 and is likely to exceed 100,000. In the white population of the Dominions it is between 40,000 and 50,000. In the native population of the British possessions in Africa, Asia, and Indonesia it is considerably more than 10 millions.

2. There has been a marked decline in the incidence of blindness in childhood in England and Wales during the past 21 years, but there is no evidence that there has been any substantial decrease in the older age groups. Before long the decline in the incidence of blindness in childhood will come to an end, as already about 65% of all blindness in children is due to congenital and genetic infections not readily amenable to treatment. With the rapid elimination of blindness due to infectious disease, intensive research in the problems of genetic disease, maternally transmitted infections, cataract, glaucoma, and the pathology of senescence, has become an immediate task if the incidence of blindness in England and Wales is to be reduced.

3. In the native populations of the British possessions the incidence of blindness could be reduced immediately and effectively by the organization of facilities for the intensive exploitation of the sulphonamides and penicillin in the treatment of the widely prevalent ocular infections.

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OBSERVATIONS ON OEDEMA OCCURRING DURING THE COURSE OF MACROCYTIC ANAEMIA

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"Tropical" or "nutritional" macrocytic anaemia is described in the textbooks, and Wells in India, Trowell in Africa, and a number of other workers have investigated it. Unfortunately, Service conditions render this literature inaccessible, and in this account of certain features of the disease which seem of practical importance I may be treading on ground that has already been covered. Reference to previous work is omitted from necessity, not from negligence.

The textbooks describe the disease as an anaemia, often severe, of a macrocytic type, occurring in India, Africa, the Balkans, and southern United States, among populations suffering from poor nutrition. It is often seen in pregnant women. It is accompanied by fever, diarrhoea, sore tongue, and sometimes oedema (which seems to have been referred to cardiac decompensation). Achlorhydria is said not to occur. Haemolytic and non-haemolytic types of the condition are distinguished.

Discussion with fellow officers suggests that many cases of this kind occur in South-East Asia Command; most commonly among Indian troops, less so among East Africans, occasionally among West Africans, and hardly ever among British soldiers. It is proposed to discuss a series of 38 cases among East Africans, laying stress on certain features.

Table I shows the frequency with which the signs and symptoms mentioned above were observed in this series. Anaemia of a more or less severe degree was noted in all (several patients had blood counts of less than one million red cells when they came under observation). It is not proposed to comment especially on the cytology, which conformed to the textbook description. Macrocytosis was observed in all cases, and normoblasts were seen on a number of occasions. The absence of an adequate centrifuge precluded determinations of mean corpuscular haemoglobin concentration, or of other features requiring haematocrit readings. On several occasions the cells were reported to be macrocytic and hypochromic. Sternal puncture was done in several cases. It always revealed an actively normoblastic marrow. Sickling of the red cells was never observed, nor was increased fragility, even in cases which were clinically of a haemolytic type. No neurological lesions of any kind were noted.

TABLE I

Total cases		38
Glossitis		12
Gastric HCl after histamine:		
Absent		12
Present		16
Not determined		10
Jaundice		5
Splenomegaly		12
Diarrhoea		17
Oedema and/or ascites		12

While gross anaemia can be missed only through negligent examination, it will be obvious that the other signs and symptoms, except the oedema and ascites (which will be discussed more fully) and the achlorhydria, may be attributed to a number of complaints very common in South-East Asia Command. Haemoglobin determinations alone are insufficient to exclude a moderate anaemia of this type. The only safe procedure is to count the red cells as well as to estimate the haemoglobin. At the least, a well-prepared thin film should be examined by a reliable observer. In this hospital some cases were certainly missed before this point was appreciated.

Fever is usual in the more severe cases, and may be mistaken for that of malaria or other illnesses. Diarrhoea may be attributed to dysentery or to infestation with worms or flagellates. Several cases at first labelled "non-specific diarrhoea" proved later to be macrocytic anaemia. Splenomegaly may be attributed to malaria or schistosomiasis; and indeed, in the cases under consideration, it was difficult to be sure whether the enlarged spleens observed were in truth connected with the

anaemia or with a legacy from earlier disease. Jaundice, when present, is more likely to suggest infective hepatitis than anaemia.

Prominence of one or more of these signs may lead to a wrong diagnosis unless the possibility of macrocytic anaemia is kept in mind as an underlying cause, and appropriate investigations made.

The occurrence of oedema and ascites merits special discussion. Fairley and Low (1939) attribute it to the cardiac insufficiency. It occurred in 12, or nearly one-third, of the present series of cases; in all but one, however, it did not appear until, as a result of treatment, the blood count had risen considerably. At this time other signs which could be attributed to cardiac decompensation were entirely absent.

Dietary deficiency is known to be a cause of macrocytic anaemia, and although an East African soldier's diet is probably better than that to which he is generally accustomed in civil life, it contains only 41 g. of first-class protein. The manufacture of haemoglobin involves the synthesis of protein as well as the manufacture of the iron-containing haem molecule. For every gramme of iron in haemoglobin there are some 800 g. of protein. If an individual with 6 litres of blood in his body is to raise his haemoglobin by 1 g. per 100 c.cm. of blood he must manufacture 60 g. of protein. It is not uncommon in these cases for the haemoglobin to fall as low as 6 g. per 100 c.cm., in which case to restore it to normal nearly half a kilogramme of protein

"heavy" nitrogen were introduced into the body either by injection or by feeding, the "heavy" nitrogen atoms could soon be identified in the protein of all the body tissues, including the plasma.

Once the osmotic pressure exerted by the plasma protein falls below a certain critical level fluid can no longer be held in the capillaries, and oedema or serous effusions result. If a diminution of plasma proteins does in fact determine the occurrence of oedema and effusion in these cases it should be possible to demonstrate it, and observations were undertaken with this end in view.

After the work had been planned and started, a copy of the *B.M.J.* for Jan. 13, 1945, became available. This contained a paper by Trevor Davies recording a case of oedema during recovery from anaemia. He found that the total serum protein, when the oedema was fully developed, was 4.16%, the albumin 1.8%, and the A/G ratio 0.76/1. At this time the red cells numbered 3,700,000. A month later, as a result of plasma transfusion and protein feeding, the total serum protein was 5.61% and the A/G ratio 1.5/1. The writer quotes another case of plasma transfusion in severe microcytic anaemia (J. McD. Holmes, 1944)—not available here. He also quotes Heath and Taylor (1936) as stating that for a rise in 10% of the haemoglobin level 80 g. of protein must be retained.

The equipment necessary for plasma protein estimations is not provided in the laboratory of a field hospital, and the

TABLE II.—Cases without Anaemia or Oedema

Serum	Total Protein (g./100 c.cm.)	Albumin (g./100 c.cm.)	Globulin (g./100 c.cm.)	A/G Ratio	N.P.N. (mg./100 c.cm.)	Remarks
1	8.43	5.66	2.77	2/1	28	Mixed sera for Kahn tests
2	8.53	6.38	2.15	3/1	28	" " "
3	6.96	5.84	1.12	5.2/1	47	" " "
4	5.72	3.40	2.32	1.5/1	28	" " "
154265	9.94	6.34	3.60	1.76/1	33	Oedema (slight) of legs. No anaemia. Periostitis of tibiae (inflammatory oedema)
8066	8.15	4.50	3.65	1.23/1	16	A case of schistosomiasis. Formol-gel test negative
14404	8.07	3.28	4.79	0.68/1	29	A case of schistosomiasis. Formol-gel test weakly positive (high globulin)

TABLE III.—Cases with Anaemia

Regimental Number	Date	Total Protein (g./100 c.cm.)	Albumin (g./100 c.cm.)	Globulin (g./100 c.cm.)	A/G Ratio	N.P.N. (mg./100 c.cm.)	R.B.C. (millions per c.mm.)	Remarks
*N/25528 44131	1/1/45 15/4/45	5.50 7.50	3.40 4.10	2.10 3.40	1.6/1 1.2/1	? 33	2.02 (Hb 3.6 g.)	No oedema or ascites Severe anaemia, very ill. No oedema or ascites
G/1419 40866	21/6/45 25/4/45	6.07 4.77	3.26 2.89	2.80 1.88	1.16/1 1.5/1	19 28	1.64 2.59	R.B.C. rising. Oedema + + +, ascites + + +. Pleural effusions
40866	6/5/45	6.01	2.58	3.43	0.75/1	28	2.34	Oedema + +, ascites + +. Pleural effusions
N/22916	29/4/45	7.81	2.90	4.91	0.59/1	27	3.10	Oedema +, ascites + +
N/22916	9/5/45	5.92	2.07	3.85	0.54/1	19	4.94	Oedema + +, ascites + + +
N/22916	30/5/45	7.55	3.02	4.53	0.66/1	24	4.26	No oedema. Some ascites, but much less than before
N/22916	19/6/45	7.08	3.00	4.08	0.73/1	11	4.48	No oedema or ascites
ZBK/12410	9/6/45	6.83	2.56	4.27	0.59/1	16	3.99	Ascites +
DN/22926	30/5/45	5.70	2.66	3.04	0.87/1	115	2.98	Chronic nephritis. Gross oedema and nitrogen retention
DN/22926	6/6/45	5.03	2.15	2.88	0.74/1	164	—	Determinations at intervals of a few days on a case with fever, splenomegaly, and anaemia, with some shifting dullness in flanks. Sternal puncture showed Leishman-Donovan bodies. Diagnosis therefore kala-azar
G/3112	15/5/45	6.99	2.50	4.49	0.56/1	25	—	
G/3112	20/5/45	7.04	2.35	4.69	0.5/1	17	—	

* This estimation was done at another hospital, and taken from the patient's notes.

will have to be forthcoming. Further, the protein moiety of haemoglobin is believed to belong to a class of proteins which are known to be particularly rich in diamino-acids, such as arginine and lysine, and, since there is evidence that these substances cannot be synthesized in the body, proteins which contain them must be eaten. Even so, it is possible that a good many molecules of food or body protein have to be broken down before the necessary constituents can be obtained for the manufacture of a molecule of haemoglobin. This may result, during the periods of blood regeneration, in a very heavy drain on the total protein resources, during which the plasma protein may become depleted.

It has been held that the plasma proteins do not form part of the general protein currency of the body. This can hardly be maintained in view of the work of Schoenheimer and his collaborators, who showed that if amino-acids containing

method used, based on that of Walther (1941), had partly to be improvised. Details are given in the Appendix to this paper. All the determinations were made on sera, since plasma could not be freed from cells without a centrifuge.

In the first instance a number of estimations of total serum protein were carried out. Though in several instances the total serum protein of cases with oedema was much diminished (e.g., the patient had a total serum protein of 4.9%, with gross oedema, on Dec. 24, 1944) the figures did not clearly establish the point at issue, but the figure for total serum protein may not be a sufficient criterion. The osmotic pressure of the serum depends upon the sum of the osmotic pressures of its albumin and globulin components (that exerted by fibrinogen is very small). The albumin is the smaller molecule, and is said to exert about four times the osmotic pressure of the globulin. A serum in which the albumin is decreased and

the globulin normal or raised may therefore exert a lower osmotic pressure than a serum of equal or less total protein content but containing a higher proportion of albumin. Stitt gives the following figures as "normal": Total protein, 6.3-8.0%; albumin, 3.8-5.2%; globulin, 2.0-3.5%; A/G ratio, 1.45/1 to 2.2/1.

The total colloid osmotic pressure is said to lie between 20 and 30 mm. of mercury. Oedema is said to occur if the total protein falls below 5.5% or the albumin below 2.5%. It is clear from this that fractionation of the serum protein is required if the influence of the serum protein in determining the oedema is to be assessed.

Table II gives the results of a number of control determinations on sera of cases without anaemia or oedema. In Table III are given the corresponding figures for anaemic patients, some of whom were oedematous, and for one or two other cases with oedema and anaemia due to other causes.

It will be seen from Table III that Nos. 44131 and G/1419, though grossly anaemic and very ill, were free from oedema or ascites and had serum albumin levels well over 3.0%. No. 40866 on April 24, 1945, was grossly oedematous, and his total protein was very low, although his albumin was only just under 3% and his A/G ratio normal. A comparison of these three cases shows that the A/G ratio, taken alone, has no particular significance (see also No. 14404 in Table II, in which the A/G ratio is low because of increased globulin). No. N/22916 had moderate oedema and ascites on April 29. It was much worse on May 9, by May 30 it was improving, and it had disappeared by June 19. Two determinations on DN/22926 are included to illustrate a type of case in which oedema has long been accepted as being due to lowered serum protein; while G/3112, who had a little ascites, exemplifies yet another condition with a low albumin and a reversed A/G ratio.

The presence of oedema and ascites in these cases of anaemia has certainly given rise to errors in diagnosis. It often occurs at a stage when the anaemia is not impressive, and at which it may be missed altogether if reliance is placed on haemoglobin estimations alone. Two such cases were evacuated to this hospital with the diagnosis of hepatic cirrhosis, and in another vitamin B deficiency was suspected.

Aetiology and Complications

These are discussed together because I feel in a real difficulty of the "chicken and egg" variety. Certain conditions have been observed sometimes to accompany the anaemia, but whether they are also its cause is very difficult to determine. Table IV gives the conditions which were noted in this series.

TABLE IV.—*Conditions Present*

Ankylostomiasis	11*	Malaria	2*
Pulmonary complaints ..	9*	Scrub typhus	1
Amoebic dysentery	3	Schistosomiasis	1
Bacillary dysentery	1	None	12

* One patient suffered from malaria, ankylostomiasis, and a mild attack of pneumonia.

It will be seen that 12 of the cases suffered apparently from uncomplicated anaemia. Ankylostome ova were found in the stools of 11. In spite of the prevalence of dysentery, in only 4 of these cases was its presence established either during or before the onset of the anaemia. We can find no evidence suggesting that the anaemia was caused by dysentery; the diarrhoea from which many of them suffered was a feature of the anaemia itself, and was not caused by a bowel infection. In only two cases was the presence of malaria parasites established. (This raises a difficulty, because the majority of East African troops come from districts in which malaria is hyper-endemic, and one would expect a higher parasite rate. On the other hand, they were all on suppressive mepacrine.) The proportion of respiratory complaints, varying from a mild bronchitis to frank pneumonia, was high. One patient was admitted with pneumonia and developed a macrocytic anaemia while in hospital.

Case DT/1574.—On admission (21/12/44): Temperature 103°, pulse 120, respirations 32. Crepitations at both apices; no frank consolidation. Not clinically anaemic. Given sulphapyridine.

26/12/44: T. normal; chest clear. Sulphapyridine stopped.

27/12/44: Moderate fever; looks pale. Hb 54%, R.B.C. 2,750,000, C.I. 0.99. Slight anisocytosis. W.B.C. 10,975 (P. 67%, E. 12%, L. 16%, M. 5%).

30/12/44. T. 100°. Weak and listless. Weil-Felix and Widal, negative.

5/1/45: R.B.C. 1,830,000, Hb 38%, C.I. 1.05. Anisocytosis, poikilocytosis, polychromasia; myeloblasts and normoblasts present; no sickling.

8/1/45: Put on high protein diet and vegemite.

21/1/45: Reticulocytes 5%.

23/1/45: Hb 82%, R.B.C. 4,630,000. Slight anisocytosis and poikilocytosis. W.B.C. 3,400.

26/1/45: Fractional test meal shows free HCl.

6/2/45: Hb 96%, R.B.C. 5,020,000, W.B.C. 4,100.

It is a pity that a red cell count was not done on admission. However, the blood counts of Dec. 27, 1944, and Jan. 5, 1945, illustrate the development of the anaemia. Large hypochromic red cells were present. The response of this case was particularly rapid. The impression gained from this and other cases is that though respiratory complaints do not cause the anaemia they may precipitate and exacerbate it.

That ankylostomiasis is potent in preventing recovery from anaemia was suggested by two cases in which ankylostomes were found and treatment was given soon after admission. After a time the anaemia failed to improve and further stool examination again revealed eggs. A second "worming" was followed by improvement in the blood count, and no more ova were seen. In face, however, of the number of cases in which neither ankylostomiasis nor any other disease was found, it is difficult to make a case for a causal relationship between the anaemia and any of the infections commonly seen in this theatre.

Of the total cases 31 were evacuated from forward areas, and 7 were admitted from L. of C. units. It is likely enough that the hardships of a particularly strenuous campaign would precipitate the condition in men already predisposed. But these figures may be misleading. At the time when almost all the sick and wounded from the East African Forces came through this hospital, all but a handful of troops were in forward areas. Later, when the division was out of the line, the bulk of the sick were diverted to another evacuation route, and were not seen here.

No apology is made for labouring the above points. In this, as in other instances, prevention is more important than cure. There is danger lest loose statement to the effect that anaemia is "always" due to malaria or ankylostomiasis may blind us to more important underlying conditions such as inadequate diet. In any case it is thoroughly established that the anaemia ordinarily caused by malaria and ankylostomiasis is characteristically of the microcytic type, and therefore different from that here described. To expect a man who has had a diet poor in first-class protein since he was weaned to maintain or replenish his haemoglobin in the face of hard campaigning conditions, with or without added infection, may be to expect him to make bricks without straw.

Treatment

Of this series one patient died from an intercurrent attack of cholera. Three were evacuated (when improving) because of pressure on hospital bed-space, and one was graded Category C on account of an old chronic synovitis of the knee. All the remainder have been returned to duty in Category A except five who are still under treatment. Some of those discharged still had achlorhydria, though three, originally achlorhydric, were found to secrete HCl when reviewed after return from the convalescent depot. Those with persistent achlorhydria all had normal blood counts, had put on weight, made no complaints of dyspepsia, and seemed so well that, rightly or wrongly, the finding was ignored and they were returned to duty. It seems, therefore, that the treatment given may be regarded as satisfactory in a long-term sense. But it has proved very slow: the average duration of treatment (including time at the convalescent depot) was 105 days; the longest 175 days, the shortest 58.

It is stated (Fairley and Low, 1939) that the condition responds to marmite, to liver, to liver extract by mouth equivalent to 1 lb. of liver a day, and to parenteral administration of liver extract. In the haemolytic type the doses of these substances may have to be very large. These authors say that before the introduction of liver therapy the mortality rate was around 40%. Moore, Vilter, Minnich, and Spies (1944), working on a series of cases in America, found that these

responded to parenteral liver and to beef muscle by mouth, and further showed that the following substances were without effect: niacin, thiamine, riboflavin, calcium pantothenate, pyridoxine, inositol, para-aminobenzoic acid, and choline.

In the present series the supply of therapeutic substances was too irregular to allow of more than a "shot-gun" regime of treatment. The diet contained about 4,000 calories. There were approximately 165 g. of protein, of which 38 g. were contained in meat (muscle), 22 g. in liver (when available), 31 g. in milk (including cheese), and 12 g. in eggs, giving 103 g. of first-class protein. (Anyone familiar with the supply position of the forward hospitals will realize that not infrequently one or more of these items were not available.) In addition, each patient received 2 to 4 oz. of vegemite (marmite substitute) and 9 multivite tablets per day. Each received an iron preparation twice daily, and for a time 2 oz. of dried yeast. For a considerable period no liver extract for injection was available, and that eventually supplied was of Indian manufacture and doubtful potency. A quantity of liver extract for oral administration was available for some time.

In all, eight patients received liver by injection. In two only was a reticulocyte response observed. Two patients showed a reticulocyte response on vegemite. Eight patients had one or more blood transfusions because of their precarious condition. Three of these were previously given at other hospitals. More would have been given at this hospital but for the difficulty of persuading East Africans to act as blood donors. However, several patients whose condition seemed very precarious, but from whom blood had to be withheld for the reason mentioned, made a good though slow recovery without it.

Treatment of Oedema and Ascites

Five of the oedematous patients received intravenous infusions of reconstituted serum. This treatment was followed by a diuresis, and in three cases the oedema and ascites began to clear up a few days later. In the other two it appeared to have little effect. It is felt that this line of treatment should perhaps have been followed more vigorously. The difficulty was, first, that the amount of serum available was limited, and a good supply had to be conserved for surgical emergencies; secondly, that the giving of serum was usually followed by a sharp rise of temperature and a good deal of malaise and discomfort. The water used to dissolve it had been bottled for some time, and one would have felt happier in preparing fresh pyrogen-free water; no glass still was, however, available. A pint of serum will contain about 40 g. of protein, of which about 22 g. will be albumin; this would raise the serum albumin by about 0.5%.

In the presence of a large effusion the question of paracentesis arises. The fluid contains protein which is for the time being out of circulation, though if the effusion is naturally absorbed it will presumably become "current coin" again. If, however, part of it is removed by paracentesis, and is replaced again (as so often happens) by further effusion, protein will be irrevocably lost to the body. As an example, on May 28, 1945, 2,570 c.cm.—a very small part of a huge ascites—was removed from the abdomen of No. 40866. This contained 93 g. of protein, of which 40 g. was albumin. The removal of this fluid made little difference to his comfort, and in a few days he was as distended as before. Subsequently (June 19) the oedema and ascites began to diminish, and had all disappeared in a few days. Earlier, N/22916 had had 3,640 c.cm. withdrawn at the cost of 32 g. of protein (which in this case was all globulin). He "filled up" again very quickly. Subsequently the whole effusion was absorbed. It is thought that, unless really serious embarrassment is being caused, effusions in these cases are better left alone.

Conclusion

In writing this imperfect account I have made use of some data—e.g., the first figure in Table III—contained in the documents of patients transferred from other hospitals, and, in certain cases, of the notes of other M.O.s (particularly those of Capt. R. A. Shanks, R.A.M.C., of this hospital). I am greatly indebted to Major A. B. Raper and his staff, especially S/Sgt. M. Fitch, R.A.M.C., for all the haematological examinations, for routine pathological examinations, and for facilities for carrying out the serum protein determinations.

Summary

A brief account is given of 38 cases of tropical (or nutritional) macrocytic anaemia among East African troops. The aetiology of the condition in S.E.A.C. is discussed.

Particular attention is paid to the occurrence of oedema and effusions. They were observed in nearly 30% of cases, and appear to coincide with the regeneration of red cells rather than with the acute stage of the disease.

A number of estimations of serum protein have been performed. Their significance is discussed.

APPENDIX

The method used for serum protein determination was based on that of Walther (1941) for total serum of plasma protein. 0.02 c.cm. of serum of plasma (measured by means of a haemoglobinometer pipette) is digested with 0.25 c.cm. of sulphuric acid and 0.5 c.cm. of saturated potassium persulphate. The digested mixture is made up to 40 c.cm., nesslerized directly, and compared in a colorimeter against standard ammonium sulphate solution. In the original method a standard correction is applied for non-protein nitrogen, since it is estimated along with the protein nitrogen. In this work boiling-tubes, a spirit lamp, sulphuric acid, persulphate, and Nessler's solution were available, and the laboratory still fortunately yielded ammonia-free water. There was, however, no colorimeter. Fortunately, the commanding officer had brought with him a Lovibond tintometer, and the 0–100 mg. blood-urea disk. These disks are, of course, made for use under the precise conditions laid down by the makers and with Nessler's solution made to a certain formula. These conditions could not be fulfilled, so urea, dried in an improvised desiccator, was weighed out, dissolved, and the solution containing known amounts of urea treated exactly as the serum would be, and used to standardize the disk in terms of mg. of nitrogen per c.cm.

The serum total nitrogen was then determined as described by Walther. After digestion the volume was made up to 25 c.cm. and the nitrogen estimated in the comparator. Dilution, which was achieved by mixing the digest with ammonia-free water in the comparator tube, keeping the final volume 5 c.cm., was carried out if required.

Non-protein nitrogen was estimated by mixing 2 c.cm. of serum, 2 c.cm. of distilled water, and 2 c.cm. of 10% trichloroacetic acid, filtering, and digesting 1 c.cm. of the filtrate with 0.1 c.cm. of sulphuric acid and 0.2 c.cm. of persulphate, and making the final volume 10 c.cm. Nesslerization and comparison were carried out as described above. This is not very satisfactory: the colour comparison is often hindered by cloudiness in the tube, due probably to the precipitation of inorganic phosphate. If, on the other hand, this is avoided by greater dilution, the colours (with normal N.P.N. levels) become too pale to be read. With this procedure, of course, the amount of filtrate digested is equivalent to 1/3 c.cm. of serum, instead of 1/50, which is used in the method for total protein. Fortunately, the protein equivalent of the N.P.N. is small, and the inaccuracy involved is not material.

It is worth noting that if the N.P.N. is high the difficulties mentioned disappear, since greater dilution can be used, and the method is then a quick and accurate one (e.g., Table III, No. DN/22926).

The fractionation of albumin and globulin was carried out on the usual principles: 0.2 c.cm. of serum was mixed with 6 c.cm. of 22% sodium sulphate (which was stored in the incubator) and the mixture allowed to stand at 37° C. No filter paper fine enough to filter off the precipitate was available, but by careful pipetting sufficient clear supernatant fluid could be obtained to carry out protein determinations, 1 c.cm. of fluid (equivalent to 1/31 c.cm. of serum) being digested as before. The blank tube contained appropriate amounts of sodium sulphate, sulphuric acid, and potassium persulphate. The result represented albumin nitrogen + N.P.N. The albumin was calculated from this and the globulin obtained from the figure for total protein by difference. All determinations were made in duplicate. Unless the duplicates were in good agreement they were repeated.

It will be appreciated that these methods were used from necessity, not from choice. The employment of so little as 0.02 c.cm. of serum implies an enormous dilution factor and a corresponding exaggeration of random errors. Distillation after digestion is always preferable to direct nesslerization, because of the presence of interfering substances. The lack of a colorimeter was a very severe handicap.

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