# A STUDY OF THE BLOOD PROTEINS AND THEIR ALTERATION IN DISEASE.

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C O N T E N T S.

## Introduction.

The view of the ancients that the blood was the vital part of the body, a view that gained expression in the system of humoral pathology, in which an attempt was made to ascribe all morbid processes of the human body to variations in this fluid, naturally led to an eager study of its composition in health and disease. The application by Lavoisier in 1780 of accurate scientific methods of measurement and analysis to vital processes, and his famous declaration that "La vie est une fonction chimique," opened up a new era in medical history, so that it is not surprising that the first half of last century saw the publication of many painstaking researches on the chemical constituents of the blood, and the alterations that might occur in disease. By 1850 however, the death knell of humoral pathology had been sounded, and the doctrine that disease depended rather on modifications in the processes of cells than in the fluids which nourish them had been firmly established. This teaching, which owed much at its inception to Virchow's work, has resulted in the huge system of cellular pathology as it is now known, a system which although it has brought great advances in medicine, has led thought too much the other way, has resulted in our pathology books belying their name and becoming text books of morbid anatomy, and has brought about an almost total neglect of the chemical processes of the body in health and disease. The last ten years, however, have seen the pendulum slowly swinging back, and the biochemist with ever improving means at his disposal is stepping forward to take his place along with the bacteriologist, immunologist, and morbid anatomist in the fight against disease.

The history of the researches on the serum proteins reflects these changing view points as to the main factor in the causation of disease. Whilst the middle part of last century saw many inquiries into the normal quantities of the serum albumin and globulin, and into the changes brought about in the amounts in various diseases, the interest in the subject evidently

waned, for little mention is made of these bodies in the literature at the commencement of this century. Perhaps the cessation of the practice of bleeding for all complaints, and the consequent fact that blood was not so available for examination, partly accounted for this. It is only since about 1912 that the study of the serum proteins has been revived, since when there has been a considerable amount of work done to determine their function in the organism, and the part they may play in disease.

So we find that Gamgee (1) writing in 1880 gave a very full account of the subject, M. Foster (2) in his physiology in 1893 estimates the total proteins as being 8.9% of the serum, and mentions that the relative quantities of albumin and globulin are very variable. Writing at the commencement of this century, however, Metchnikof (3) in his "Immunity in Infective diseases," apart from noting the fact that the antitoxins are precipitated with the globulins, takes no notice of the changes in the serum proteins in infections. Ehrlich (4) in his "Studies in Immunity," and later in his work with Lazarus on Anaemia does not refer to these bodies at all. Wm. Hunter in his careful account of Pernicious anaemia, omits any mention of the proteins of the blood; and Buchanan (5) in his book entitled "Blood in Health and Disease" published as late as 1909 has nothing to say about the subject. In the last few years it is remarkable to note that standard works like Starling's and Halliburton's physiology text books, and Adami's, MacCallum's, and Beattie and Dixon's pathology books only state baldly that the blood contains serum albumin and globulin. The best modern accounts of the work are given by Hammarsten, (6) Luciani (7), Robertson (8) and Macleod (9), but these are all very brief, and in the first two cases rather erroneous.

In the account given below, an attempt has been made to indicate the views held concerning the serum proteins at different times, and to give in more detail the modern work that has been done in attempting to solve the problem of these substances.

The account of the estimations of the serum albumin and globulin made at different times by various workers is by no means complete, for Rowe (10) published a very full bibliography of the subject in 1916. Rowe in his account, however, only gives, practically, a list of the values for the total proteins, serum albumin and globulin, found by different workers up to that date, with the references. He does not enter at all into the subject of the functions of the blood proteins, or of the use that may be made of their estimation in medicine. Whilst most of the references to old work made below will be found in his article, yet in some instances literature has been found to which he evidently did not have access. Moreover, much work has been done since his articles appeared, so that the account below is in no way simply traversing the ground again, but is rather supplementary to his article, looking at the subject from a different view point, and also giving new work done since that time.

## Historical Summary

## of work done on the Serum Proteins up to the present day.

It is difficult to place with certainty when the presence of serum albumin and globulin was first recognised in the blood. Hewson (11) who was Hunter's partner from 1762 to 1772, and gave some of the lectures for Hunter, did much valuable experimental work on the blood. From his writings, however, it appears that the main problem of the day, and the one towards the solving of which most of the experiments were directed, was how the clot was formed from the circulating blood, what constituents of the blood entered into the clot, and in what form these bodies existed in the fluid blood. It was known that the serum was coagulable by heat, and various other agents, as alcohol and the salts of mercury, and further that the part left after coagulation, known as the "serosity," contained all the mineral salts of the serum. But little attention seems to have been given to the coagulable matter, except that Hewson compares it to the pellicle which forms on milk when it is heated. There is a shrewd remark in a book published before Hewson's time in 1754 by R. Brookes (12) that the blood serum coagulates like white of egg which, he adds, it resembles.

It must be remembered that in the years before Hunter and Hewson, argument had waxed strong as to whether the red corpuscles entered into and assisted to form the clot, and it was only during the time of these two great teachers that the fact that the red cells have nothing to do with the clot, became universally recognised. The fibrin we know today was variously styled "coagulable lymph," "plastic lymph," or "gluten", to mention a few of the terms, and it was known that this substance was the same whether it appeared in coagulated shed blood, in the clot on wounds, in emboli in the vessels, or deposited in flakes in inflammatory processes. Cullen (13) in Edinburgh, from his book for the students in 1777, taught that this "gluten" did not all solidify in the clot, but that some remained as the coagulable material of the serum. That is, he thought that the

serum protein and the fibrin were identical. This was soon corrected, for Blumenbach (14) in Germany writing in 1817, remarks that the coagulable part of the serum is albumin, and says that this substance used to be confused with fibrin.

In 1830, the first records of quantitative estimations of serum protein commence. These measurements were at first of the total proteins only, for even when it was known that the protein existed in two or more forms, it was all believed to be albumin modified in different ways, and it was all grouped together under that name. At this time Prévost and Dumas (15): (1821 - 1823) and Berzelius (16) of Thenard, did work of great value by estimating the average composition of healthy blood, and thus establishing a standard with which the blood in disease could be compared. The latter writer in 1831 records the average value of the total proteins in normal serum, as being 8%. The next twenty years saw the publication of innumerable estimations of the composition of the blood of various animals as well as of men, with comparison of the alterations in the quantity of the constituents, in the two sexes, during starvation, in persons of different temperament, and in diseased conditions. Simon (17) in his "Animal Chemistry" gives a very good account of the researches by the workers of this time. He notes the results of Denis, Lecanci, Nasse, and Andral and Gavarret, who obtained results for the total proteins ranging from seven to nine per cent, and compares them with his own results of 7.5 - 7.7% for healthy persons. He thought from the results of these workers and himself that sex, age, and temperament all exerted an influence on the composition of the blood, albumin being present in greater quantities in females. The great work of the time, however, was done by Becquerel and Rodier (18), who investigated the chemistry of the blood in health and disease in a very thorough manner. These writers found that the normal value for the serum proteins was 8%, and that in chlorosis and plethora, there was no alteration, whilst in nephritis and cardiac disease

with oedema, endocarditis, typhoid, acute rheumatic fever, pneumonia, secondary anaemias, and pulmonary tuberculosis, they were lowered. For the time at which they worked, these two men were remarkably accurate, and noted their many facts which are thought today to be new discoveries. For example, they showed that the cholesterol in the blood was increased in old age, a fact which has been rediscovered by many workers of late years.

But as yet little was known of the nature of the serum proteins or the part they played in the bodily economy. For Liebig (19), one of the fathers of our modern organic chemistry, and who stood alone in his day, writing of the blood in 1842, states that it contains "fibrine" and "albumin," and that these bodies were isomers of one another. He held that the nitrogenous constituents of blood, of muscle fibre, and of vegetable food were isomers, and could all be converted into albumin, and that the reverse process also was possible. He thought that "albumin" and "fibrine" could be changed into one another, and into muscle fibre, and this latter substance back into them. To use his own words "The true starting point for all the tissues is consequently albumin; all nitrogenised articles of food, whether derived from the animal or vegetable kingdom, are converted into albumin before they can take part in the process of nutrition." So that the serum protein was then believed to exist for nutritive purposes, a view, which will be seen later, has been held even of recent years, but which modern work seems to have finally disproved.

Liebig also describes the discovery of a substance by Mulder which was believed to be the basis of all nitrogenous organic bodies, and which had been named "proteine." It was thought that substances such as albumin and fibrin were formed by the union of this "proteine" with various inorganic substances. From now onward this term commences to be used in the literature, gradually assuming the significance which is attached to it today.

In 1843 an outbreak of cholera in Dorpat provided C. Schmidt (20) with material to work on, and he found that the serum proteins were increased in severe cholera, and also after the use of hydragogne cathartics, and he confirmed Becquerel & Rodier's (18) findings that the total protein is diminished in nephritis. He recorded the value of the proteins as being in cholera 15.3% and in nephritis 4.4%. He adopted the explanation, which is still held today, that the blood loses fluid in the former states, and becomes diluted in nephritis, and so the change in the percentage composition is brought about.

For about the next 30 years, apparently very little work was done with regard to the serum proteins. Very few references exist to any estimations made during this period. Claude Bernard (21) in 1859 in his lectures, states that the serum albumin is difficult to measure, but that as it varied so widely both in health and disease, not much importance could be given to alterations in the quantities present. This great physiologist held, though, the modern view that the proteins of the blood do not exist as such in the circulating fluid, but combined together, but the deductions from the experiments he did to prove this, were, in the light of present day knowledge, erroneously drawn. Leven (22) in 1873 found that the normal value of the total serum protein was 7.9%, but that from a patient with scorbutus it was 7.6%. Then A. Schmidt (23), working on the coagulation of the blood, incidentally made some observations on these bodies, and it is with his work that the first definite recognition of the globulin and the albumin fractions appear. Unfortunately this scientist, although he did very valuable work on clotting, work which stands to this present day, made two mistakes with regard to the serum proteins. His mode of separating the globulin fraction was by dialysis, which, of course, only gave him the euglobulin, so that his estimations of this substance were very low. Thus he found ox blood to contain 0.4% of globulin, whereas the true figure is somewhere about 2.2%. Secondly he thought that the globulin, or "fibrinoplastic substance," as

he termed it had some part in changing the fibrinogen into fibrin.

These errors were recognised by Hammarsten (24), who in 1878 pointed out that all the globulin was not precipitated by A. Schmidt's method, and discovered that saturation with magnesium sulphate threw down all the globulin, whilst not affecting the albumin. Incidentally, in his invaluable researches into the coagulation of the blood, he followed up and extended Schmidt's discoveries, but corrected Schmidt's error of supposing that the globulin acted as a fibrinoplastic substance. Hammarsten (24) published the following estimations of the serum proteins in normal cases:-

| Serum | Total Proteins | Albumin | Globulin |
|---|---|---|---|
| Horse | 7.26% | 2.67% | 4.56% |
| Ox | 7.49% | 3.33% | 4.17% |
| Rabbit | 6.22% | 4.43% | 1.79% |
| Man | 7.62% | 4.52% | 3.10% |

From this it can be seen that except in the case of the rabbit, his values for globulin are far too high, but it should be remembered that the rise in these bodies produced by infection was not known in those days, and consequently this fallacy was not guarded against.

Mention must be made at this stage of Gamgee (1) in England who evidently had a wide knowledge of the work done on the blood, and carried out work on it himself. In his book published in 1880, he gives a very good account of the subject, particularly of the work of Hammarsten and A. Schmidt, and describes the various methods then extant of estimating the serum proteins. Unfortunately he does not state any quantitative results he himself obtained.

During the remaining years of the nineteenth century, comparatively little work was done, the main workers in this field during the period under review being von Jaksch, Limbeck, and Pick. After Schmidt and Hammarsten's work, however, the importance of estimating the globulin and albumin fractions of the serum protein was recognised, so that the results published from this time

on, practically all show the variations in the relative amounts of these two bodies, as well as in the total amount of proteins present. In 1382, Hoffmann (25) found the normal value of the serum protein to be 7.4 to 7.8%. Estelle (26) and Csatary (27) in 1880 and 1891 respectively, recorded the results they found in cases of "nephritis," the latter finding that the globulin was increased in this condition. Mya & Niglezio (28) investigated the albumin-globulin ratio in various diseases, and found that the globulin was increased in pneumonia, angina pectoris, tetanus, nephritis, and diabetes. They also estimated the same ratio in different transudations and exudations, and found that the alterations in the serum vary directly with those in the transudations. Hoppe Seyler (29) determined the total proteins in a case of melanotic sarioma, and found that they were lowered. This author (30) published a new method of carrying out this estimation, using alcohol as the precipitating agent for the protein in the serum. He also noted (30) that an approximate value for the amount of serum protein could be obtained with a clear serum by the polarimeter. Several workers at this time sought the effect, if any, of starvation on the amount of protein in the blood. Thus Panum (32) found a diminution in fasting animals, and concluded that part at least of the serum albumin must be considered as nutrient material. Bruckhardt (33) and Tregel (34) found in dogs and snakes respectively that the globulin is increased relative to the albumin in starvation, but Howells & Savioli (35) showed that their work was erroneous. A small amount of work was done by Halliburton (36) on the composition of human serum in disease. He stated that the globulin was increased in acute fevers, and connected this with the accompanying leucocytosis. He also estimated the normal values of the proteins in Hen's serum (37), but later work has shown his figures to be erroneous.

After all these isolated researches, a more complete survey of the subject was undertaken by Limbeck and Pick (38) in 1383, and investigations made into the changes both in the total

protein and in the serum albumin and globulin in numerous diseases. These investigations were undoubtedly spurred on by the work of von Jaksch (39), who just at this time published the results of his estimations of the serum protein in a host of diseases. The method used by von Jaksch was to find the amount of nitrogen in the serum, and to calculate the albumin present indirectly from this by multiplying the result obtained by 6.23. It will be obvious at once that such a method gives too high results, and this was immediately pointed out by Limbeck and Pick, who in numerous experiments in various diseases showed the difference in the results obtained for the value of the total serum protein when using von Jaksch's method, and when estimating the protein by precipitating it and weighing it. These writers from a study of the results both of other workers and themselves felt certain that the total amount of protein in the serum was comparatively fixed, although the serum albumin and globulin might vary considerably as regards one another. They further investigated this latter question, and obtained first that the normal relations were albumin 62 - 33% and globulin 17 - 38%. Similar estimations carried out on the serum of persons suffering with disease gave them varying results, but as, on the whole the globulin was generally raised in quantity relative to the albumin, they concluded that the facts indicated that the globulin is more resistant in the serum.

The only other researches in this subject during the remainder of this period that are of much interest are those of Freund (49) in 1895 who found that the percentage of globulin in nephritis varied from 25 - 33 per cent., whilst in pernicious anaemia it was 33 per cent., and of Seng (41) who in 1899 found an increased percentage of globulin in animals immune to diptheria. Szontagh & Wellman (42) also found that with immunization the total proteins were slightly increased, and their work was repeated and confirmed by Butjagen (43) in 1902.

The investigations concerning the serum proteins made during the present century may be conveniently considered in two groups, namely, those made up until the years 1914 and 1915, and those performed since that time. The former researches were chiefly carried out on the Continent, the latter in America. This division, although quite arbitrary, aids one in grasping the history of the subject. For as will be emphasised later, any work done before 1915, must be examined very carefully, and discounted to a certain extent for the following reasons. Firstly, all the methods employed in those times, to estimate the serum proteins, as has been shown again and again by various workers, were inaccurate. But, as with one worker using the same technique for all analyses, the same error occurs each time, so, although the actual values obtained may not be true, nevertheless the comparisons made of the proteins in various conditions by this worker may reveal any alterations faithfully, as for example a diminution in the total proteins in a case of nephritis with the advent of oedema. Secondly, it was not fully realized that infections caused a rise in the amount of globulins, and even when it was recognised, this fallacy was, and often could not, be excluded. Even in the present day, when we have the additional help given by bacteriological examinations, by radiographic examinations, and by direct instrumental examination of various membranes, as by the urethroscope and cystoscope, it is often impossible to detect the presence of an infection in a patient, which subsequent events show to have existed all the time. How impossible it was, then, for an old time observer, in noting an increase in the globulin percentage, to say definitely what this was due to. So that the old records must be considered very cautiously, and even modern work, where an increase in the globulin albumin ratio is shown, must be charily accepted, unless it is shown that all necessary care has been taken to exclude the possibility of a complicating infection. Even then, knowing the difficulty of doing this in the human body, it seems necessary if one wishes, to prove actually that a rise in globulin does take place in a particular condition, to observe this rise in a

large number of cases.

To return to a review of the actual researches carried out in the earlier period mentioned above, the work of Erben (44) must first be considered. In a series of papers published during the years nineteen hundred to nineteen hundred and five, this investigator reviewed the existing literature on the chemistry of the blood, and then gave his own findings for the normal amounts of its organic and inorganic constituents. He further investigated the serum proteins in a number of diseases, and amongst his results it may be noted that he found the total proteins lowered in a case of pernicious anaemia, but with no rise in the amount of globulin. In nephritis and carcinoma, the serum protein was also lowered, and he noted an increase in the globulin in various infections, for which he offered the explanation of Gottwalt (45) that the globulin was more resistant to the toxins than the albumin. The increase in the amount of globulin in different infective processes evidently attracted notice at this time, for several workers turned their attention to it, and their results can be briefly reviewed at this point. In 1900, Atkinson (46) showed that the antitoxic power of the serum was associated with the globulins, a most important discovery for from it has grown the methods of preparation now employed to obtain anti toxins for therapeutic purposes. In nineteen hundred and two, Jakoby (47) found an increase in the euglobulin in animals immurized to castor oil, and Joachim (48), besides showing an increase in the globulin in human serum in many diseases, demonstrated that the same phenomenon happened when a horse was immunised to diptheria. This work was followed up the next year by Moll (49) who injected various proteins and killed bacteria into animals, and found that this produced a rise in the globulin, which he associated with the increase in the immune bodies. He revived the suggestion put forward by A. Schmidt (23) that some of the globulin may possibly arise from the leucocytes. Similar experiments were carried out in 1904 by Longstein and Meyer (50) who showed an increase in the

globulins in the sera of immunized and infected rabbits, and this work was confirmed by Muller. Glaessner (51) repeated Moll's work in 1905 and confirmed it, but offered the explanation that globulin was more resistant to ferment action than albumin to account for the increase of the former body in infectious diseases.

The work of Vadala (52) who published the estimations of the serum proteins in two cases of anaemia from ankylostomiasis, finding the albumin to be 7.8% and the globulin 2.4%, and of Grentt, Gilbert, and Chiray (53), who found the serum proteins decreased in liver insufficiency, may be mentioned here in passing, but the next important group of researches into the subject concern the behaviour of the serum proteins in Syphilis. The estimation of the total proteins in this disease had already been made in 1902 by Jolles and Oppenheimer (54), who found no alteration, but the first mention of the determination of the albumin globulin ratio was made by Winternitz (55) in 1908. He found an increase in the amount of globulin. This increase was also noted in the same year by Klaussner (56), and by Sachs and Altmann (57), the latter believing that the Wasserman reaction was due to the increased globulin. Elias, Neubauer, Porges and Salomon (58) also thought that the substance causing the Wasserman reaction belonged to the globulins, which bodies, they noted, were increased in syphilis. Noguchi (59) investigated the problem, and he thought that the increased globulin ran so nearly parallel to the Wasserman test that, for a time, he suggested the estimation of globulin as a test for syphilis. Gay and Fitzgerald (60), however, were unable to obtain such definite results as did Noguchi, and in 1911 Muller and Hough (61) could establish no relation between the increase in globulin and the result of the Wassermann test. Rowe (10) and others of more recent years have definitely settled this question, and shown that the Wasserman reaction is not due to the increased amount of protein, but their work will be considered later.

In concluding the survey of the work of this period, the researches of Epsteen in America, and of Reiss and his co-

workers with the refractometer on the Continent, remain for consideration. The former (62) in nineteen hundred and twelve, thirteen, and fourteen published three papers dealing with the chemistry of the blood serum, included in which were estimations of the serum proteins in various conditions. He concluded from his results that the globulin was increased in amount in "cardiac diseases associated with decompensation and serous effusion," in "pulmonary or respiratory affections of inflammatory or non- inflammatory origin (pneumonia, emphysema, polycythemia)" in "diabetes mellitus," "parenchymatous nephritis," and in "localized infections of the kidneys." He found that the globulin content of the serum is normal or diminished in the following diseases, namely, "simple achylia gastrica," "tuberculosis," "diabetes insipidus," "chronic interstitial nephritis," and "prostatic hypertrophy." In "minor surgical cases" the protein content was also normal. Unfortunately, in none of his cases does he give any indication as to whether the presence of a complicating infection was excluded. He does not even say whether Wasserman tests were performed, so that it would be certain that the rise in the amount of globulins reported was not due to syphilis. It may be recalled here that Epstein has published further articles (63) since, in which he has used the estimation of the serum proteins to guide him in the diagnosis and treatment of certain cases of nephritis. In fact, he has separated off a class of nephritis in which there is a heavy loss of protein in the urine, accompanied by a very low amount of serum protein with a large increase in the globulin albumin ratio, and termed such cases "nephroses." Oedema is generally a feature of these cases at one stage or other of the disease. Epstein thinks that the low protein content of the blood is partly, at any rate, due to the heavy loss through the kidneys, and believes that the disease is unlike a true nephritis but is comparable rather to diabetes, being a disturbance of the protein metabolism. Further, the low amount of serum proteins, he thinks, by lowering the osmotic pressure of the blood, plays a large part in causing the oedema.

It was for this reason that he introduced the well known "Epstein diet," which consists of a menu rich in protein, saying that so the protein content of the blood is increased, and the oedema consequently decreases. Although in practice his diet in the right class of case, is often successful, the reasoning on which it is based is fallacious. For, as will be shown in the series of cases presented below, there can be a very heavy loss of protein through the kidneys without any alteration in the serum protein values, and conversely the total serum protein may be greatly lowered and the globulin-albumin ratio increased without any albuminuria. Further, as has been shown by many experimenters, feeding of any description, makes no difference to the amount of protein in the blood. English workers, foremost among whom Maclean (64) must be mentioned, have demonstrated that it is rather the metabolic products of the rich protein diet that cause the decrease in oedema, and in those cases where such a diet is successful, equal results may be obtained by the administration of urea.

The thorough and careful investigation of the serum proteins in health and disease published by Reiss in 1913, however, overshadows all other work done in this field at about that time. After the introduction of the use of the refractometer to determine the total proteins quantitatively by Strubell in 1900, this method was perfected by Reiss in 1902, and thereafter used by him to estimate these bodies in physiological and pathological states. All his work together with that of the many investigators, who had also used this method since its introduction, amongst whom may be mentioned Strauss, Böhme, Oppenheimer, Sandelowsky, and Widal, is summed up in his admirable account. It must be borne in mind, however, in considering Reiss' work, that his method was only for estimating the total serum protein; he could not estimate the albumin and globulin separately. He tried to evolve a way of doing this with the refractometric process, but by a mischance - using too strong acetic acid to precipitate the proteins and obtaining hydrolysis - failed, and concluded that it was

impossible. Further, his values for the total proteins were obtained on the assumption that the non protein constituents of the blood were constant in quantity. After estimating the refractive index of the whole serum, he subtracted a certain fixed number representing the index of the non protein constituents, and so obtained that of the protein elements. Reiss recognised himself that in uraemia, because of the retention of certain bodies in the blood, this method of procedure as given above rendered his results valueless. It is known now that in many other conditions besides anaemia, a high content of non protein constituents may exist. Consequently in all such cases his method is liable to give erroneous results. An additional error was introduced into the values he obtained as follows (66). The refractive index of a one per cent solution of globulin is much greater than that of a similar solution of albumin. So that the refractive index of a solution containing one per cent of protein made up of a mixture of albumin and globulin will depend on the relative amount of these two bodies present, and will vary as they do. Now the serum protein of the blood is just such a mixture and to assign a fixed value to the refractive index of a one per cent solution of it, when the albumin-globulin ratio in it is always varying is an impossibility. Yet this is what Reiss attempted to do, and so introduced a further error into his analyses. But, if these errors in Reiss' work are recognised, and allowed for, his results are still very valuable. From a study of the existing literature, and from his own researches he came to the following conclusions, namely, that the total serum proteins were the same in amount in capillary, venous, or arterial blood. Venous stasis, he found increased the proteins, as did muscular work. Feeding caused no difference, but by drinking large amounts of water, he was able to lower the amount of protein by 0.2 - 0.6%. Perspiration did not alter the percentage present, but blood-letting lowered it more than could be accounted for by the mere loss of protein in the shed blood, and during the first and second days of menstruation, it was also lowered. He found that the normal

value of the total serum protein was 7 - 9%, but for persons lying in bed, lower figures were not unusual. He estimated the percentage of protein present at various ages from birth to 18 years and found that it gradually rose from 6.7 - 8.5% in the first two years and thereafter is practically constant. In investigating the changes in disease, Reiss reasoned that the amount in the blood might be less owing to a real loss of protein, or from a dilution of the serum, and similarly an increase might be accounted for in two such ways. So in all his cases, he kept a chart of the body weight and of the chloride excretion. If there was an increase of body weight, he assumed that there was a retention of fluid in the body, and any decrease in protein found would be due to a dilution of serum. As the protein might increase, decrease, or remain stationary in amount, and the body weight could do likewise, it will be seen at once that there are nine possibilities in any case. Reiss recognised this, and noted which occurred in any particular case, and the deduction he might thereby draw. Working along these lines, he investigated cases of diabetes, nephritis, heart disease, acute infecticus diseases, pernicious anaemia, syphilis, and various intestinal disorders. In nephritis, he found that there was a diminution in the total amount of protein due to a retention of water, and this "oedematous state of the blood" was present before oedema was clinically manifest, and also after it had disappeared. In cardiac cases, he concluded that there was no alteration until compensation failed, when there was a decrease in the amount of protein, again due to dilution of the serum. Reiss strongly refutes Strauss' contention that the blood proteins are much lower in nephritic than in cardiac cases, so much so that diagnostic help may be obtained from this point. In acute infectious disease, during the period of fever, a retention of salt and water was found, with a consequent lowering of the serum protein. In pernicious anaemia, however, there was a true loss of protein, not due to dilution of the serum, and in syphilis there was a rise in protein during the incubation and

secondary periods. Reiss advocated the clinical use of serum protein estimations especially in the treatment of nephritis. He thought that the diet and administration of drugs could be regulated by observing the amount of protein in the blood. A falling indicated an "oedematous" state of the blood, and gave a warning that oedema would soon appear clinically if measures were not taken. Again, when a patient had had dropsy, and this had disappeared with treatment, Reiss believed that the examination of the blood proteins revealed the true state of the patient, and if the test showed that the proteins were still low in amount, it meant that oedema was liable to recur and treatment should still be persisted with. Reiss' assumption, however, that, because in certain conditions, he found an increase in the body weight with a decrease in the amount of serum protein, and therefore the blood was diluted, needs reinvestigation. For work with modern means of estimating the blood volume leads one rather to believe that the body keeps the amount of circulating fluid fairly constant, so that before accepting his conclusions as proven, it would be well to see what results are obtained in progressive estimations by the "vital red" or "carbon monoxide" method, in cases, say, of acute infections or nephritis.

It will be well to pause here and consider the actual methods employed in all the researches that have been dealt with so far.

In some of the latter, as has been indicated already, the use of the refractometer was employed. The best technique in the application of this instrument to the determination of the serum proteins was that developed by Reiss. The weaknesses of his procedure, and the errors introduced by them, have already been discussed, so that there is no need to dwell on them again here. All the other estimations were carried out by precipitating the proteins by various agents, and then finding out the amount of precipitate. The globulins were thrown out of solution by salts such as magnesium sulphate and ammonium sulphate, whilst the total proteins were thrown down by such

reagents as acetic acid, and alcohol. The amount of protein precipitated was then estimated either by filtering, and weighing the residue on the filter paper, or by obtaining the amount of nitrogen in the precipitate by Kjeldahl's method, and calculating the amount of protein from this. The technical difficulties involved in these procedures render them very inaccurate when dealing with the small quantities of material met with in analysing blood sera, and moreover the tediousness of the methods and the relatively large amount of blood required for the estimations precluded repeated observations being made on any one subject, and so an accurate picture of any variations being obtained. These considerations vitiate the work of all observers up to 1914 whether their results were obtained by refractometric or other methods. Moreover, as has been stated above, in considering their work in detail, where estimations of the serum proteins in only one or two cases of a disease have been published, the question as to whether the results have not been rendered inaccurate by a complicating infection, has always to be reckoned with. In spite of this, these researches are valuable today, in that the conflicting results can be weighed over, and the balance used to provide guidance for future work, or even as accessory evidence in favour of more recent findings. Moreover, a study of the old experiments reveals the pitfalls that must be avoided if we wish to obtain accurate analyses today.

Reiss' work, which has been reviewed above provides a very adequate summary of the knowledge which had been gained up to that date as to the quantities of proteins normally present in the blood and the variations that might occur in disease. In addition, it can be seen that it had been almost universally found that the quantity of globulin increased in infections. But although the various theories already mentioned had been put forward to account for this rise, practically no experiments had been carried out to confirm any of them, nor had any attempts been made to see what purpose in the bodily mechanism this phenomenon served, if indeed it did any. The views concerning the functions

of the serum proteins that held most sway at the time under discussion were that these bodies had nutritive duties to perform. Abderhalden (67) supposed that, as foreign proteins are not tolerated in the circulation, the products of the digestion of proteins in the intestine were resynthesized by the intestinal cells, just as the fats were. But unlike the fats, the proteins formed did not resemble those of the food whence they came, but were the serum albumin and globulin, the only proteins that are borne in the blood stream. Thus he thought that the blood proteins were the form in which nitrogenous material was carried to the various parts of the body for anabolic processes. Abderhalden was arguing on the assumption that the amino acids are not absorbed as such, an assumption which has since proved to be false. Luciani (68) in his "Human Physiology" published in 1911, which still ranks as a standard work, so thoroughly and completely did he deal with his subject, held similar views. He states that the serum proteins are tissue-forming substances, of which the albumin is the true form, and the globulin a different modification produced by cell metabolism. He quotes Miescher and Burchhardt as having actually shown that the globulins of the blood increase during hunger whilst the ablumin decreases, and says that these same facts and the explanation of them have been confirmed by many experiments by Fano and his pupils, Ducceschi and di Frassinetto.

The survey of the latter of the two groups into which we separated the work done in the present century may now be entered on, and the reason why this arbitrary division was made can be briefly stated at once. It has just been seen how the observers before this time laboured under the disadvantages of imperfect methods of analysis, and also how the question of infection vitiated their results. From the commencement of the period now coming under review, both these obstacles that had been blocking the progress of knowledge in the subject were removed for the following reasons, Firstly, two simple accurate methods of estimating serum proteins, only requiring small amounts of serum for an analysis, were devised. Secondly, it was fully

recognised by all investigators at the outset that most infections, if not all, brought about an increase in the globulin-albumin ratio, so that the errors that this might introduce were as far as possible guarded against.

In 1915, Robertson (69) published his refractometric method of estimating the proteins of human sera. This investigator had, in the preceding years, been working with the refractometer, determining the refractive indices of different substances when dissolved in various solvents, and in 1912 had shown how the proteins of ox serum could be measured by means of the instrument. In his paper (70) at that time, he critiscised Reiss' technique, and showed where the values assumed by that author were wrong. The method even as described then was simpler and more accurate than any other in vogue at that time, and was used immediately by its inventor and several men working under him to carry out investigations on the percentages of albumin and globulin present in the sera of various animals under normal and fasting conditions. Robertson (71) determined the blood proteins in the ox, horse, rabbit, and rat, and noted that there was an alteration in the albumin-globulin ratio during starvation, the direction in which this alteration proceeded apparently varying with the species. An increase in the total protein with starvation was also observed. Woolsey (72), Thompson (73), and Briggs (74) carried out similar investigations on other animals. Their work was revised in 1916 by Jewett (75), who corrected their results by determining the percentage of non protein constituents usually present in the sera of the animals that had been experimented on. He substituted these values in the work of the preceding investigators, in place of the constant value which had been employed by them. These researches are interesting in that they are the pioneer work done with the new method, and furnished normal values for the serum proteins of many animals. The conclusions formed, however, as to the influence of food on the quantities of albumin and globulin

present, were drawn from too small a number of experiments, and were later found to be erroneous. Two other interesting researches were carried out at the time that the work just mentioned was going on. The first was by Wells (76), who investigated the serum proteins in rabbits aged from three weeks to a year. He found that the percentage was low in the young animal, and gradually increased withage up to the time when the rabbit was 5 months old, at which time it assumed a constant adult value. He also tried feeding the rabbits on different diets, but could detect no change in the amounts of the serum proteins as a result. The second piece of work referred to was done by Buck (77), who kept animals under anaesthesia with ether or chloroform for prolonged periods, so that albuminuria began to appear, when the globulin-albumin ratio was found to rise, far more than could be accounted for by the loss of albumin in the urine.

The method as used in these researches, however, was further simplified and improved, and published (69) in 1915 as an accurate, practicable method of analysing human serum for the quantities of non-protein constituents, albumin, and globulin present. It had the advantage of only requiring one and a half cubic centimetres of serum to carry out the test, and thus could be performed at frequent intervals on a patient, without causing any undue loss of blood. In experimental work, too, where the effect of some procedure on the serum proteins was under investigation, the quantities of these bodies could be ascertained from hour to hour without leading to an anaemic state of the animal in use, and so producing the complicating factor of haemorrhage.

The principles of Robertson's procedure may be briefly described as follows. The refractive index of the serum to be analysed is determined. One cubic centimetre is then taken and boiled with a like amount of dilute acetic acid to precipitate all the protein. The clear fluid so obtained, containing only the non protein constituents of the blood, is taken and its refractive index read. From this the percentage of non protein constituents can be determined. The globulin in half a cubic centimetre of serum is then precipitated by half saturation with

ammonium sulphate, and the fluid left, which contains the serum-albumin and non-protein constituents has its index read off on the refractometer. By subtracting the refractive index of the non-protein constituents obtained above, that of the serum albumin is obtained, and hence the quantity of this body present. Then knowing the refractive index of the whole serum, it is a simple matter by subtraction to obtain the amount of serum globulin.

The second new method referred to above was of later origin than the one just mentioned. It was originally published by Van Slyke and Cullen (78) in 1920. These workers did not have a refractometer available, and so had to develop some other technique. They followed Robertson in using Ammonium Sulphate as the precipitating agent for the globulins, saying that in spite of the criticism that had been levelled against the use of this reagent, they were agreed from Robertson's work that it was the most satisfactory salt for the purpose. In their article, they discuss briefly the various methods of estimating the protein after precipitation. Washing and weighing the precipitate they dismissed as too inaccurate, and nephelometric methods were debarred for a like reason. There remained the estimation of the nitrogen in the precipitate, and the calculation of the protein present indirectly from this. It must be remembered that with the introduction of colorimetric procedures, the analysis of a substance for the amount of nitrogen present has become simple, rapid, and accurate, thus removing the drawbacks under which previous workers with this form of analysis, laboured. Hence the reason for Van Slyke and Cullen adopting this particular method. The process in brief then, consisted of precipitating the fibrinogen in a measured quantity of oxalated plasma with calcium chloride, estimating the nitrogen in the precipitate, and so calculating the amount of protein present. The globulins and fibrinogen were then thrown down in another quantity of plasma by ammonium sulphate, the ammonia got rid of by boiling the precipitate with magnesium oxide, and the amount of protein determined as before. A third quantity of serum was treated with trichloracetic acid,

to precipitate all the protein, and the nitrogen in the filtrate due to the non-protein constituents estimated. Finally the amount of nitrogen in the whole serum was found. From these results, the amount of albumin, globulin, and fibrinogen, can be calculated.

This method had the disadvantages of requiring twenty cubic centimetres of serum in order to carry it out. Also, the getting rid of ammonia from the globulin precipitate by boiling with magnesium oxide was found, for technical reasons, to be difficult in practice. So Howe (79) in 1921 published a modification which utilised only 2.5 c.c. of serum, and in which the necessity of getting rid of the ammonia was done away with by substituting sodium sulphate as the precipitating agent for the globulins. The trouble with this salt is that all precipitations have to be carried out above 34 degrees C., which means keeping all solutions and apparatus in an incubator or hot room, whilst the test is being performed, but Howe did not seem to think that a hardship. He stated that he found three critical strengths of the sodium sulphate solution, at each of which he got down a definite portion of the globulin, so in his technique, the globulin is always estimated as euglobulin, pseudo globulin 1, and pseudo-globulin 11. Howe and several workers after him have since used this technique to carry out researches which will be spoken of later. For quickness and simplicity, however, it can be seen from the foregoing description, that this latter cannot compare with the refractometric method, and it certainly is no more accurate.

To turn from the methods used to the work accomplished with them, the researches of several workers who used the refractometric method to attempt to solve the problem presented by the rise of globulins in infections must be considered. It has been seen above that this rise had been recognised for a considerable time, and it had also been known that in many cases the immune bodies produced were associated with the globulin fraction of the serum proteins. Arising from this, there were two schools of thought; one who held that the antibodies are a form of blood

globulin, and the other who considered that the antibodies are only carried down in association with the globulin. The first attempt to solve this problem was made by Hurwitz and Meyer (80) in 1916. Already in this year, Righetti (81) had made a study of the albumin-globulin ratio in the serum of rabbits immunized against typhoid, and found that the globulin rose in quantity, whilst the albumin decreased, during the period of immunization, the total protein remaining practically unaltered. In animals who had been highly immunized, the globulin was found to be raised, and on reimmunization the globulin-albumin ratio was found to first decrease, and then rise, but this rise was not due to an increase in the percentage of globulin, but a decrease in the amount of albumin, and a lowering of the total amount of protein. Hurwitz and Meyer investigated the question much more thoroughly. They determined the amount of serum proteins and of immune bodies at frequent intervals in rabbits whom they infected with various organisms, and immunized against different bacteria. They found that the rise of globulins produced in no way ran parallel to the production of immune bodies, or to the leucocytosis, and further that if the immunization was carried out carefully with well graded doses, no rise in globulin need be produced. So, too, in a very mild chronic infection, the rise in globulins was exceedingly slight. They also injected bacterial toxins into rabbits, and found that these alone gave a rise in the globulin albumin ratio. Following this, they injected "aleuronat" intraperitoneally, thus producing a non-bacterial inflammation, and here also a rise in the percentage of globulins was noted. It is rather doubtful, however, whether these last experiments can be interpreted as they wish, for in their reports of the autopsies of the rabbits treated thus, it is noted that there was intestinal paralysis present, with coprostasis, and considerable enteritis, so that evidently there was a secondary bacterial infection produced by the procedure. Hurwitz and Meyer concluded that the evidence brought forward by their results was against the view that the immune bodies were a form of blood globulin. They thought, further,

that a bacterial invasion was not necessary for the rise in the percentage of globulin, but that this rise might best be explained as being due to the metabolic disturbances, particularly the acceleration of metabolism, brought about by the infective agent. In the following year, E.S. Schmidt and C.A. Schmidt (82), working together, confirmed the observations noted above, namely, that the increase in globulin in infections is not due to the leucocytosis. They did this by producing an increase in the white count by the injection of various pure proteins, causing a decrease by administrating benzol, and estimating the number of white cells and the amount of serum protein simultaneously. No relation between the two could be noted. These workers found, like Hurwitz and Meyer, that immunity could be produced without an increase in the percentage of globulin taking place. Schmidt and Schmidt argued that if the rise in globulin was due to the formation of antibodies the injection of non-antigenic bodies, as pure casein, should cause no alteration in the albumin-globulin ratio. It had already been reported by Wells (83) that the injection of gelatin, which is non-antigenic, caused such an alteration. So they tried the effect of injecting non-antigenic bodies, but could find no rise in the percentage of globulin. It was shown later, in 1913, by Clark (84) that Wells' work was incorrect, for Clark injected gelatin and could obtain no alteration in the relative amounts of the serum proteins.

The idea put forward by Hurwitz and Meyer, that the cause for the increase of the percentage of serum globulin was to be found in metabolic disturbances, was made the subject of an investigation in 1917 by Hanson and McQuarrie (85). Cervello (86), and Breinl (87) had, seven years before, subscribed to this notion, stating that a rise in the amount of globulin in the blood was found after administration of antipyrin. These two workers, like Hurwitz and Meyer were labouring under a delusion that albumin could be converted into globulin, owing to the preposterous assertion by Moll (88) that he had performed this feat by the action of warm alkali, apparently obtaining the glycine necessary

for this conversion out of the air. Undoubtedly from their articles, this idea had led all these workers astray in their thinking. Hanson and McQuarrie administered antipyrin to rabbits, and also acetanilide and paramido phenol, but found that these bodies had no effect on the serum proteins. They then employed quinine and sodium cacodylate as substances retarding metabolism, and thyroid extract as accelerating metabolism, and here again found no effect produced on the relative proportions of the serum proteins. Hanson (89) further studied this question by alternately starving and feeding animals, and estimating the serum proteins day by day during the experiment. He argued that if any disturbance of metabolism would cause the rise in the globulin percentage, surely the one thus produced by starvation would. Hanson carried out a large number of experiments, and he was unable to find any alteration produced in the albumin-globulin ratio as a result of the fasting or feeding. So taking this in conjunction with his other work, it seems that the explanation of this phenomenon must be sought elsewhere than in the idea that it is produced as a result of disturbed metabolic processes. Hanson carried out one other research (90) on this subject. He noted that inall the experiments in this subject that had been performed up till then, the antigenic bodies used had been such that the antibodies produced in response to them were carried down with the globulins on precipitation. Trypsin on the other hand, produces antibodies in the albumin fractions. So Hanson tried immunising animals to this body, but he only confirmed the previous work that immunity is non-dependent on the concentration of the serum proteins.

Whilst all this work had been proceeding on experimental lines on animals, Rowe (91) had been using the refractometric process clinically to study the serum proteins in health and disease. He prefaced the account of his results with a most complete bibliography of the subject. This worker first of all determined the normal values of the serum proteins in healthy adults, and his results are those generally accepted today. They are as follows:-

| | | | |
|---|---|---|---|
| Non protein | 1.1 - 1.3 per cent. | Average | 1.2 per cent. |
| Serum Albumin | 4.6 - 6.7 " " | " | 5.6 " " |
| Serum Globulin | 1.2 - 2.3 " " | " | 1.9 " " |
| Total Serum protein | 6.5 - 8.2 " " | " | 7.5 " " |

Globulin forms from 16 - 32%, of the total protein, averaging 25%. Rowe found that venous stasis increased the quantity of protein in the blood, so that in taking a specimen a tourniquet must not be applied to the limb for longer than one minute. He used this fact to show that the Wassermann reaction does not depend on the increase of globulin in the serum, as had been stated by some observers. He then investigated the alterations produced in the serum proteins in disease. Hiscontributions on this subject form the most important data that have been made available for many years, and it is necessary to consider his findings at some length, as they are practically the only modern estimations of the serum proteins in disease available. Rowe analysed the blood for its protein content from a series of cases of syphilis and pneumonia, and in both diseases found that the globulin was increased. The total protein, however, remained about normal in syphilis, whilst in pneumonia it was decreased, for which Rowe gave Reiss' explanation that there was a dilution of the blood from a retention of water. He then made determinations in a series of infections of different types, as, to choose a few at random, pyelitis, endocarditis, abscess of the lung, tuberculosis, and typhoid. He found that serum globulin was definitely increased inall infections except acute tonsillitis, typhoid, and certain mild infections as chronic bronchitis. It must be borne in mind, however, that only once case of each disease was examined in this series. Nephritis was made the subject of an intensive study, and in all types there was found to be an increase in the relative amount of globulin, but the total quantity of protein varied, becoming low with the onset of oedema. In cardiac decompensation, the total proteins were found to be low, and the globulin normal or slightly raised in amount. A number of cases of arteno sclerosis and diabetes were also investigated.

In the former condition, the globulin was sometimes slightly increased, but in the latter, not at all, unless there was a complicating infection. The total proteins in both were normal. Three cases of pernicious anaemia showed a lowering in the total amount of protein in the blood, but the globulin percentages were normal in each case. In addition cases of hyperthyroidism, goitre, haemophilia, pellagra, obesity, lead poisoning, chronic intestinal disorders, and neurasthenia were examined, but showed no deviation from the usual serum protein values. Rowe showed by charts how in any illness the values of the non-protein constituents, and of the proteins, returned to normal with recovery, and suggested that the use of such charts might help in determining prognosis and treatment. He also thought that in nephritis, much useful information as to the condition of the patient could be gained from a study of the serum proteins.

Much interest, clinically, too, attaches to Alder's (92) work in 1913. This writer states that from his researches he concludes that there is practically no variation in the composition of the blood whether it is venous or capillary, that sex causes no difference, and that in any individual there is scarcely any alteration from day to day. He also found that ingestion of food and muscular activity cause no alteration in the protein content.

In this same year further experimental work on the blood proteins in animals was carried out by Hatai(93), who investigated the amount of these bodies present at different ages in thealbino rat. His researches were repeated in 1919 by Toyama (94), who agreed with Hatai that the increase in the proteins with age followed the growth curve. This and the succeeding year, however, was marked by the publication by Whipple and his co-workers of a most complete and thorough investigation of the function of the serum proteins. It has been stated before that at various times the view had been held that the serum albumin and globulin existed as nutritive bodies, in fact, that the part played by glucose in carbohydrate metabolism was similarly ful-

filled by these bodies in protein metabolism. From the researches that have been described already, it will have been seen that the effects of fasting and feeding on the serum proteins had been investigated on numerous occasions with conflicting results, but that the most thorough and extensive work of Hanson had upheld the view that these bodies were unaffected by diet. Kerr, Hurwitz, and Whipple (95) wished to see whether they could find any evidence that the blood proteins might be regarded as intermediate products between food proteins and tissue proteins. To commence with, they studied the serum proteins at frequent intervals in dogs fed on different diets, and also starved for varying periods, but could find no alterations from these procedures. They then lowered the quantities of the serum proteins in the blood of the dogs by an ingenious procedure which they termed "plasmapharesis." This consisted of bleeding the dog, and immediately returning the washed corpuscles suspended in Locke's solution. Thus no anaemia was produced, nor was the blood volume altered, In their first experiments, it was found that by repeating the "plasmapharesis" several times in a day, the serum proteins could be lowered to any desired level. In all cases frequent red cell counts were made, and estimations of the relative volumes of the red cells and plasma carried out by the haematocrit. By this means, it can be seen in all their records that there was no diminution in the erythrocytes, nor was the blood volume altered, so that these complicating factors were avoided.

When the blood proteins had been lowered by these means, their regeneration was studied under different conditions. It was found in all cases that there was a quick immediate increase in the first twenty four hours, generally amounting to about one per cent, but that after this, the regeneration was a slow process, it taking several days for the normal level to be reached again. The effect of diet on this process was studied, and it was found that regeneration occurred even when the animal was starved, but more slowly than when fed with meat.

The authors argue that just because starvation and

feeding produce no change in the quantities of serum proteins, it cannot be assumed that these bodies are not intermediate metabolic products. For glucose is just such a product, yet under like conditions it is kept marvellously constant. When, however, by plasmapharesis, the proteins are reduced in amount, and then on a meat diet, they do not immediately recover, it seems fair to assume that a similar role to that of glucose cannot be assigned to the serum proteins. Kerr, Hurwitz, and Whipple then tried the effect of injury to the liver cells. They found that this produced a fall in the amount of serum albumin and globulin. Moreover, when plasmapharesis was performed on dogs in whom the liver cells had been injured, or on whom an Eck's fistula had been performed, the regeneration of the serum proteins was greatly delayed. They pointed out also, that the curve of regeneration of the serum proteins presented many points of similarity to that obtained for the regeneration of liver cells by Whipple and Sperry. So they concluded that the liver plays some part in maintaining the level of the albumin and globulin in the blood. An attempt was made to confirm this later by perfusion experiments, but failed through technical difficulties.

These investigations were continued by Whipple, Smith and Belt (96), who improved the technique of the plasmapharesis. In the experiments just described several successive bleedings were employed to lower the blood proteins. Instead of this, a certain quantity of blood was drawn off from the dog, and at the same time an equal volume of washed red cells suspended in Locke's solution was run through a vein. It was found that thus any desired lowering could be obtained in a few minutes. The experiments on regeneration were repeated, and the previous observations confirmed. The immediate rise in the serum proteins after plasmapharesis, noticeable in fifteen minutes, and even more so in twenty four hours, with the slow following increase, were still more marked in these experiments.

It had been noticed in some of the experiments that shock was produced by the plasmapharesis. This was made the sub-

ject of further investigations. It was found that if a dog was taken and the level of the serum proteins lowered, at a certain point shock supervened. The level at which this occurred varied tremendously in different dogs, but in any one dog seemed very fixed, for if a mild degree of shock were produced in an animal by lowering to a certain value, and the animal was then allowed to recover, on subsequent occasions it was found that decreasing the proteins to the same level again always produced the same degree of shock. The grade of shock produced varied directly with the amount the serum protein was lowered. Decreasing the total quantity of protein present in the blood below one per cent always brought on fatal shock. The nature of the shock produced clinically resembled "surgical shock," and at the autopsies on animals in whom fatal shock had supervened the picture was similar to that produced by anaplylactic shock. When in performing the plasmapharesis, dialysed serum was used instead of Locke's solution to suspend the corpuscles, no shock occurred. So they argued that the shock was not due to the returned corpuscles or to any part of the technique, but to the lowering of the serum proteins.

Further experiments were carried out in which the effect ofplasmapharesis combined with liver injury was studied. In preliminary experiments, it was ascertained that a certain lowering of the serum proteins produced no shock, and the animal was allowed to recover. Later "plasmapharesis" of similar extent was performed, combined with an injury to the liver cells which it was known would not by itself cause any ill effects. The two together, however, were found to produce fatal shock. It was not due to cell injury for similar experiments performed with injury to the kidney, pancreas, and small intestine, had no such result. Whipple, Smith and Belt explained this by saying that the injured liver cells are more susceptible to other injurious agents than the normal cells, and can not stand the change in the environment produced by the lowering of the proteins. Under these conditions, they form poisonous bodies which are carried over the body and produce fatal shock. These workers conclude

from their investigations that there is strong evidence that the serum proteins are stabilizing and protective factors, and are essential environmental factors of the circulating blood in relation to the tissue cells. This, they think, may be the most important function of these bodies.

Researches along quite different lines were carried out by Howe (97) in 1920, using the modification, devised by him, of Cullen and Van Slyke's method, which has already been described. It has been noted in the preceding pages that several workers have found the serum proteins in various animals to be low in amount at birth and gradually increase with age. Howe analysed the serum of newly born calves, and determined that the globulin was exceedingly small in amount, owing to the euglobulin and pseudo globulin 1 fractions being missing. Immediately after the administration of colostrum, however, the globulin content of the blood increased. Moreover, Howe was able to demonstrate that this increase was proportional to the amount of globulin in the colostrum; where the colostrum was poor in this protein, the rise in the calf's serum was not marked, but where globulin was present in large amounts in the colostrum, after its administration, a correspondingly large increase was noted in the calf's serum. In animals who did not receive colostrum, the globulin took three or four weeks to reach the same level as that in the calves, who had received this substance. Lewis and Wells (98) performed similar experiments on infants. These writers point out that colostrum differs from milk in the high content of globulin it contains. Moreover, it has been shown by Woodmann (99), by means of Dakin's racemization method, and by Wells and Osborne, by immunological reactions, that the globulin of colostrum and serum globulin are identical. The albumin in colostrum however, although it is the same as lactalbumin is absolutely different from serum albumin. Casein likewise has no counter part in the blood. Lewis and Wells go on to point out that Famulener (100) showed in 1912 that there is a transfer of immune bodies from the mother to the infant at birth through the

colostrum, and this work has since been confirmed by Reymann (101), Howard and Ely (102), and Little and Oraitt (103). Lewis and Wells analysed the sera of six new born infants, and found the globulin to be very low in amount, the euglobulin fraction being practically missing. In three infants who had had colostrum, the globulin was much increased whilst in one who had not, the globulin was comparatively low. So their results for infants agree with Howe's for calves. They subscribe to the opinion that the colostrum presents to the new born mammal a concentrated solution of serum globulin, which carries antibodies from the maternal blood, and that it is of the highest importance that the new born child should receive the colostrum.

A clinical use of the estimation of the serum proteins has been made in America in connection with Syphilis. It was found by Tokuda (104) that the rise in the amount of the globulins and the results of the Wassermann test paralleled one another. It was found possible to use progressive estimations of the percentage of globulin present as a quantitative measure of the efficacy of the treatment being employed, so that it was possible to gauge what curative effect any particular remedy was having on a patient.

In concluding this review of the literature of the subject, two other important aspects of our knowledge concerning the blood proteins must be briefly referred to. It has been mentioned already that Claude Bernard in the middle of the last century believed that the proteins existed in some combined form in the blood stream. Important and most interesting work was done on this question by Hardy in 1905, and many of his experiments were afterwards repeated and confirmed by Robertson. Hardy (105) was able to show that the serum albumin and serum globulin do not exist in ordinary solution in blood, but bound up in a protein complex. So that when these bodies are isolated, and estimated, they probably represent fractions split off from complex compounds present in the plasma. Unfortunately very little attention has been paid to this work, and no further attempts have been made to elucidate the matter.

The question as to whether the serum albumin and globulin each consist of a mixture of several proteins has also not been discussed as yet. Because the albumin is separatable into a crystalline and non-crystalline portion, and because the globulin similarly can be split up into numerous fractions, it has been held by many workers, that not two, but many proteins existed in the blood. The delicate test introduced by Dakin (106) however, who found that each protein, had its own peculiar "racemization" curve, when applied to the blood proteins, revealed that the separate fractions of globulin were all chemically identical. It may be noted here that it has been shown by Quinan and later by Chick, that the so called "insoluble globulin" of the blood differs from the remainder of the globulin in that it is combined with a lipoid. This fact probably accounts for its different precipitation. So that probably the view that there is only one globulin and one albumin present in the plasma is correct.

From the foregoing review of the literature, the state of our knowledge at the present time concerning the serum proteins, then, may be summed up as follows. Two proteins, serum albumin and globulin, exist in the serum, probably in the form of a protein complex. At birth, the total quantity of protein present in the serum is low in amount, apparently owing to a deficiency in the globulin fraction. With age, the amount gradually increases until it reaches the adult value. In health, the amounts of these bodies present are very constant. They exist in equal quantities in arterial, venous, and capillary blood, and there is no variation with sex. Muscular exercise apparently makes no difference to the amount of these bodies present in the blood, nor does food. Even starvation, although it may increase the total quantity of protein slightly, causes no alteration in the relative amounts of the albumin and globulin present. Apparently they form an important constituent of the fluid environment of the body cells, and if the amount present in the blood is lowered too far, changes fatal to the organism are brought about. It would appear that the body keeps a small reserve store of these substances, which can be rapidly poured out into the blood stream in case of

emergency, but beyond this the formation of new serum protein is a slow matter. Which particular organ or organs manufacture it is not definitely known, but there is some evidence to show that the liver does so.

Concerning the functions of the serum albumin and globulin, but little can be said. They assist the circulation mechanically to a certain extent by maintaining the colloidal character of the plasma, so that excape of fluid from the vessels by diffusion is hindered. Furthermore they cause a viscosity of the blood, which, by increasing the resistance offered to the flow through the vessels, helps to maintain blood pressure. The theory that they are nutritive substances receives little support from the assembled facts. They undergo certain alterations in disease both in quantity and in the relative proportions of each present. This alteration particularly takes the form of a rise in the globulin-albumin ratio in most infections. The reason for the phenomenon has been eagerly sought for, but as yet no satisfactory explanation has been offered. It has been seen that this rise is not due to the formation of immune bodies, to the accompanying temperature or leucocytosis, or to the retarded or accelerated metabolism. On the other hand, there is some slight evidence in favour of the view that the phenomenon is due to an alteration of the permeability of the cells of the body, and it is in this direction apparently that an explanation must be sought.

# EXPERIMENTAL RESULTS.

It will have been gathered from the review of the literature just concluded, that, since the introduction of a satisfactory technique for estimating serum proteins, laboratory experiments, having for their end the elucidation of the problems presented by these bodies, have been carried out in all directions. On the other hand, clinical research in this subject has been sadly lacking. Excepting the work of Epstein and Rowe, practically nothing has been done. But as it has always been found in medicine that laboratory and clinical research must go hand in hand, if any problem is to be solved satisfactorily, it follows that there is an urgent necessity for this latter in the case of serum proteins. Our knowledge of the behaviour of these bodies in disease is very fragmentary. In some diseases no attempt at their estimation has been made; in most of those diseases where such investigations have been made, the results have rested on the examination of only a few cases. It was felt that it would be profitable to increase both the number and variety of cases examined, and particularly to combine the analyses of the sera with careful clinical examinations and records of the cases.

The present investigation was undertaken, therefore, with the view,

1. To estimate the serum proteins, if opportunity offered, in diseases where no such analyses had been made with modern methods.

2. To obtain supplementary results in diseases where our knowledge of the behaviour of the serum proteins rested on only one or two analyses.

3. To ascertain whether, by a study of the variations in the quantities of the serum proteins in various diseases, and by the correlation of these results with the clinical findings, some light might be thrown on the origin or function of the serum proteins, or, may be, a clue obtained to direct further research.

4. In particular, to extend the observations concerning the rise in globulins in order to learn whether this happened universally in diseases due to a bacterial invasion; or whether an infection could occur without this rise, and further to note whether this phenomenon occurs in any condition where an infecting agent is absent.

5. To ascertain, in the event of the rise in globulin being peculiar to infectious diseases, whether this fact can be utilised as an aid to diagnosing the infective origin of obscure conditions.

6. To contrast the estimation of the non-protein constituents of the blood, by the refractometric method, with that of the blood urea, with a view to ascertaining whether the former test will show retention of bodies from kidney insufficiency before the latter.

The estimations of serum proteins given below were all done by Robertson's refractometric method. This is fully described in Appendix B, but it may be added here that the procedure was simplified somewhat as follows. In place of the thick walled tubes made from glass tubing mentioned in the author's directions, it was found that for the precipitation of all the protein by acetic acid, long narrow bored test-tubes served quite as well, and as these could be purchased cheaply, the time and labour expended in making the above mentioned tubes was saved. Similarly for the precipitation of the globulins by ammonium sulphate, small narrow bored centrifuge tubes filled with corks were found much more convenient, as these could be placed on the high speed centrifuge, and the globulins separated very rapidly. Otherwise Robertson's description was followed closely.

At the commencement of the work, two experiments were carried out on all sera. After six months, it was found that identical results could be obtained from the two experiments, the inaccuracies that are liable to creep in in this work having been eradicated apparently by then. All the results obtained in this preliminary period have been disregarded, only those obtained since the technique was perfected being given below. In many of these estimations whenever sufficient serum was obtainable, two experiments were carried out in order to keep a check on the accuracy of the work.

The main source of error was found to be in precipitating the globulins. Variations in the saturation of the ammonium sulphate solution, due to differences of temperature on succeeding days, at first gave considerable trouble, but this was satisfactorily overcome by obtaining a saturated solution at 15 degrees C., and then decanting this from the crystals, and keeping it for use out of contact with solid ammonium sulphate. Further, it is important when the serum is added to the saturated ammonium sulphate solution for the purpose of precipitating the globulins, to ensure that the two liquids are quickly and thoroughly admixed. Otherwise parts of the mixture have a high concentration of ammonium sulphate; here the albumin will be precipitated as well as the globulin and so too high values for this latter substance be obtained

A Pulfrich refractometer was used for the readings of the refractive indices of the solutions. Early in the work an Abbe' was tried, but this, although much simpler to work with, did not give accurate enough results. The readings with the Pulfrich, however, were found to be very simply and easily made, provided the sodium flame was satisfactory, flickering in the flame, impurities in the colour, or insufficient luminosity leading to blurring of the edge of the shadow.

As regards the obtaining of material from the patient, blood was withdrawn with a serum syringe from a suitable vein in the forearm. It is preferable to do this as long after a meal as possible, for with blood taken soon after feeding, a lipaemic serum may be obtained, and the refractive index of this is more difficult to determine. When taking the specimen it must be remembered, that if this is being obtained for estimation of the serum proteins, a tourniquet should not be applied for more than one minute. For it has been shown by Howe that with venous stasis the serum albumin and globulin are proportionately increased. The blood so obtained was allowed to clot, and immediately centrifugalised and the serum pipetted off. Hurwitz and Meyer showed in 1916 that serum so obtained, if kept cool and free from bacterial contamination, would show no appreciable change in the serum protein values after 72 hours, but that if it was left in contact with the clot, by the end of 24 hours, a marked alteration would have occurred. In the present experiments the effects on the refractive index of keeping the serum 24 hours in the ice chest were tried several times, but no appreciable difference was noted, thus agreeing with the work mentioned above. For example, the following three cases may be cited. The reading for distilled water is given in each case, from which it can be seen that in two cases no allowance for difference of temperature need be made, whilst in the third the correction for this makes the serum readings practically identical. The refractometer can only be read to the nearest minute.

| | Angle of Total Reflection taken as soon as possible. | Angle of Total Reflection 24 hours later. |
|---|---|---|
| Exp No. 1. Serum | 65 36 | 65 35 |
| Distilled water | 63 5 | 63 5 |
| Exp No. 2. Serum | 64 23 | 64 21 |
| Distilled water | 67 53 | 67 53 |
| Exp No. 3. Serum | 65 41 | 65 38 |
| Distilled water | 67 59 | 67 57 |

An attempt was also made to see whether the refractive indices of the fluids obtained by precipitating the total proteins and the globulins only from the serum altered when these fluids were left in contact with their precipitates in the cold room for twenty four hours. The idea was that if no change was observed, in cases where time pressed, the experiments might be interrupted at this point and concluded later. The results obtained were very variable however. For example, the following results may be quoted. For convenience in the table, the fluid obtained after precipitating the proteins with acetic acid will be termed "Filtrate A," and that from the precipitation of the globulins with ammonium sulphate "Filtrate B."

| | Angle of Total Reflection Read as soon as possible. | Angle of Total Reflection Read 24 hours later. |
|---|---|---|
| Exp 1. Water | 67 23 | 67 23 |
| Filtrate A. | 67 2 | 67 2 |
| Exp 2. Water | 63 5 | 63 5 |
| Filtrate A. | 67 43 | 67 50 |
| Exp 3. Water | 67 53 | 67 53 |
| Filtrate B. | 67 40 | 67 37 |
| Exp 4. Water | 67 23 | 67 23 |
| Filtrate B. | 62 41 | 62 34 |
| Exp 5. Water | 67 53 | 67 53 |
| Filtrate B. | 63 13 | 63 12 |

In some cases no change occurred, whilst in others it was quite marked. So that any thought of this proceedure was given up. In the experiments given below, in all cases the serum was separated from the clotas quickly as possible, and the determinations on it concluded within the next twenty four hours.

With regard to laking of the serum, this of course, introduces an error into the estimation. With an ordinary sodium flame and the Pulfrich refractometer, it becomes impossible to read the refractive index of the serum accurately when the laking exceeds a certain degree.

With laking of a less degree than this, i.e., so that the serum's refractive index can be determined properly, Robertson found that the error introduced by the dissolved haemoglobin was so small that it was negligible. Thus the apparatus itself prevents any errors being introduced from this source.

The accuracy of the method once the technique is acquired depends practically on the reading of the refractometer. This can be read to the nearest minute so that the percentage error in the different estimations is as follows:-

| | | |
|---|---|---|
| Error in the estimation of the percentage of | non proteins | 0.1% |
| " " " " " " " " | albumins | 0.2% |
| " " " " " " " " | globulins | 0.15% |

The estimations of the blood urea nitrogen were carried out by Folin's method, using the distillation process in preference to aeration for obtaining the ammonia, and determining the amount of ammonia colomnetrically after Nesslerization. The blood sugar values were likewise obtained by Folin's test, and the urinary diastatic test, when done, was carried out according to Cole's modification of Wohlgemuth's method.

Record of Results.

In the report given below of the results found in various morbid conditions, full accounts of the cases have been omitted, and only the facts essential to the argument have been given, in order to make the reading less tedious. All the cases are numbered, however, and by referring to Appendix A under the desired number, the record, complete as far as possible, of the patient may be found. Before going on to give the experimental results, it will be well to recall that the normal values for serum proteins accepted at present are

| | | |
|---|---|---|
| Non protein | 1.1 - 1.3 per cent. | |
| Albumin | 4.6 - 6.7 per cent. | Average 5.6 % |
| Globulin | 1.2 - 2.3 per cent. | " 1.9 % |
| Total protein | 6.5 - 8.2 per cent. | " 7.5 % |

globulin being 16 - 32 per cent. of the total protein.

1. The Proteins in Various Blood Conditions.

Examinations were made of the blood in five cases of pernicious anaemia, four cases of lymphatic leukaemia, in a case of secondary anaemia of unknown origin, and finally in a case of Banti's disease. The results in the cases of pernicious anaemia may be tabulated as follows:-

| Case No. | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent. | Total Protein Per Cent. | Albumin expressed as Percentages of Total Protein | Globulin expressed as Percentages of Total Protein |
|---|---|---|---|---|---|---|
| 1. | 1.7 | 3.7 | 2.6 | 6.3 | 59 | 41 |
| 2. | 1.6 | 4.4 | 1.8 | 6.2 | 71 | 29 |
| 2. (1 wk. later) | 1.3 | 4.6 | 1.8 | 6.4 | 72 | 28 |
| 3. | 1.4 | 5.4 | 1.2 | 6.6 | 82 | 18 |
| 3. (2 wks. later) | 1.0 | 4.0 | 2.2 | 6.2 | 65 | 35 |
| 4. | 1.4 | 4.5 | 2.3 | 6.8 | 66 | 34 |
| 5. | 1.1 | 5.0 | 1.4 | 6.4 | 78 | 22 |

It will be seen from this that there is very little, if any, alteration of the albumin-globulin ratio in these cases of pernicious anaemia, but that the percentage of total protein is low. Reiss (65) estimated the total serum proteins in two cases of this disease, and found the amount lowered. He ascribed this, not to a dilution of the serum, as in nephritic and cardiac cases, but to an actual loss of protein. Rowe (91)

also examined the blood in three cases, and found the percentage of globulin unaltered, but the total protein decreased, so that the present results confirm his work. It has been pointed out already that Reiss' assumption that there is a retention of fluid in the blood in certain cases leading to its dilution, and so to a lowering of the percentage of total protein, has never been proved. Epstein (63), on the other hand would have us believe that in one type of nephritis there is such a lowering of the serum proteins, that this causes oedema. We know that oedema may occur in advanced cases of pernicious anaemia, and it would be interesting to see the amount of blood proteins present in these cases, but unfortunately no opportunity for such an investigation occurred. There is apparently no relation between the seriousness of the condition of the patient, and the amounts of serum protein present, for cases one and two were improving rapidly, and returned home to light work, three and four were cases ill enough to be kept in bed, but whose general condition was not alarming, whilst case number five was in a serious condition and died shortly after.

A comparison of the percentage of globulin present with the white and red cell count and the haemoglobin percentage will be given later in conjunction with all the other blood cases. The results obtained in the case of secondary anaemia and in the patient with Banti's disease may now be considered. The former case (Case No. 6) was an interesting one in a married man aged forty six who had been ill for five months, complaining mainly of weakness in the legs, with feelings of giddiness, and a tendency to fall over. He was in hospital for a considerable time during which he was subjected to a most rigorous examination, which revealed a secondary anaemia with an eosinophilia, for which no cause could be found. The Wassermann test both of the blood and cerebro spinal fluid was negative. The estimation of his serum proteins revealed a large increase in the globulin fraction. The results were as follows:-

Non protein 1.3 per cent.
Albumin 2.0 per cent.
Globulin 4.0 per cent.

Total protein 6.0 per cent, of which 33 per cent. is albumin and 66 per cent. globulin. As it is well known that the globulin

is raised in infections, these figures supported the idea gained from the rest of his examination that the anaemia was due to some undiscoverable infection.

The case of Banti's disease (Case No. 7) revealed the following figures:-

| | First test. | Test a fortnight later. |
|---|---|---|
| Non protein | 1.5 | 1.2 |
| Albumin | 4.6 | 3.7 |
| Globulin | 3.1 | 3.7 |
| Total protein | 7.7 | 7.4 of which |
| of which | 60% | 50% is albumin |
| is albumin & 40% globulin | | & 50% globulin. |

Here again this is evidence in favour of the view that the causal agent of this condition is an infection of some description. But not conclusive evidence, it must be admitted. For whilst, as far as is known, the amount of globulin is raised relative to the albumin in all infections, it is not satisfactorily settled that it is not so raised in any other condition. Indeed, as has been stated in the historical account, some hold that it is not the infection but the accompanying metabolic disturbances, that produce the change in the proteins. The weight of the evidence at present available, however, is in favour of the view that the alteration is produced in infections, and in no other states.

As far as we have been able to ascertain, no estimations have been made of recent years of the serum albumin and globulin in leukaemia Rowe (91) did not include any cases of the disease in his series. Reiss (65) in 1914 reported that the total proteins were normal in severe cases of leukaemia, but apart from this no mention has been found. Fortunately four cases, all of the lymphatic type, were available whilst this investigation was being carried out, and the analyses of their sera were as follows:-

| Case No. | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent | Total Protein Per Cent. | Albumin expressed as Percentages of Total Protein. | Globulin |
|---|---|---|---|---|---|---|
| 8. | 2.4 | 2.1 | 2.7 | 4.8 | 44 | 56 |
| 8.(6 wks. later) | 1.8 | 5.4 | 0.8 | 6.2 | 87 | 13 |
| 8.(1 wk. later) | 1.8 | 3.4 | 3.3 | 6.7 | 53 | 47 |
| 9. | 1.3 | 6.2 | 0.8 | 7.0 | 88 | 12 |
| 10. | 1.2 | 3.9 | 2.5 | 6.4 | 61 | 39 |
| 11. | 1.8 | 4.2 | 2.6 | 6.8 | 62 | 38 |
| 11.(1 wk. later) | 2.0 | 3.6 | 3.1 | 6.7 | 54 | 46 |

These results are rather confusing. It appears from them that the globulin percentage is generally raised, but the number of estimations is too small to generalise on. Case Number 8 is very interesting. The patient was a married man of 68 years, who apparently had had the disease for twelve or thirteen years, for he had noticed the glands in his neck and groin slowly growing during that period. An acute exacerbation brought him into hospital, and the first estimation done two days after his entry revealed an increased percentage of globulin. With the administration of benzol, he improved, and when the next test was done six weeks later he was up and about, feeling quite well, although his white count had only dropped during this time from 256,000 to 215,000. The exceedingly low value obtained for the globulin, only thirteen per cent. caused us torepeat the test with another supply of serum but the same result was again obtained. Only a week later on increased doses of benzol, when his general condition was still improving, with the glands shrinking, and the spleen smaller, the value obtained for his globulin was forty seven per cent. The white cell count was then 187,000. The other case, number nine, in which a low globulin percentage was obtained, was also a case which was very chronic, for the patient was able to carry on his work, and felt quite well. The patient had been on benzol for some time until three months before the test when, as his white count was down to 10,000, it was discontinued. At the time of the test, the count had risen again to 116,000. These low figures cannot be attributed to the benzol for Schmidt (83) has shown that administration of this has no effect on the globulin percentage. The other estimations were all done on cases of a more severe type. Hurwitz and Meyer (80)

have shown that although the globulin percentage is generally raised in infections, if the infection is one to which there is little reaction on the part of the body, this rise is not experienced. Similarly in immunising an animal to bacteria, if the doses injected are large, the globulins are increased in amount, but with carefully graded doses, no alteration in the albumin-globulin ratio is observed. Bearing this in mind, those who hold that the condition known as leukaemia is due to an infective process, might explain the figures given above by saying that in the cases with low globulin values, there was very little reaction on the part of the body to the infecting agent whilst in the others some reaction was taking place. This matter can only be settled by much further research into the values of the serum proteins obtained in leukaemic cases, with a correlation of the results with the clinical pictures.

It is of interest to recall here that the two chief theories regarding the causation of lymphatic leukaemia are that it may be infectious in origin, or that it may be a malignant disease of the blood or blood forming organs. If further cases of leukaemia reveal a rise in globulin, this would provide additional evidence in favour of the first view, provided of course, that cases of malignancy reveal no such changes. A study of the literature showed no modern results in malignant diseases, so that an attempt was made late in the present work to investigate this matter. It is complicated, however, by the fact that only those cases can be regarded as suitable for this purpose in which examination reveals no trace of any complicating infection. The four cases of leukaemia recorded here were all searched most minutely from this point of view, especial attention being paid to the teeth, naso pharyx, and its air sinuses, and to the genito urinary system, with negative results as regards the presence of infection in all cases. Wassermann tests were also performed and gave negative results Investigations therefore, were limited to three persons suffering with carcinoma who revealed no evidence of coincident infection. The serum proteins were present in the following amounts:-

| Case No. | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent. | Total Protein Per Cent. | Albumin expressed as percentages of total protein. | Globulin |
|---|---|---|---|---|---|---|
| 12 | 1.1 | 3.7 | 3.4 | 7.1 | 52 | 48 |
| 13 | 1.4 | 1.1 | 3.9 | 5.0 | 22 | 78 |
| 14 | 1.7 | 2.0 | 3.7 | 5.7 | 35 | 65 |

The first case - No. 12 - was that of a married man aged 68, who had an epithelioma of the tongue. He felt well, and had only come to the hospital because he had noticed a swelling in his mouth. He was well nourished, and there was no ulceration of the growth, so that the possibility of a secondary infection locally was ruled out. Examination revealed no trace of any other infection. A section was taken from the growth and examined microscopically to confirm the diagnosis. With him, and also with the other two cases, Wassermann tests gave negative results. The second case (No. 13) was not such a good one for the purpose of this examination, as the patient, a single man, aged 63, was very emphysematous, and coughed up a considerable amount of phlegm each day, so that there was a possibility of a secondary infection being present in the bronchi. The patient was found at operation to have a gastric carcinoma. The last case of this series (No. 14) was a single woman forty seven years old who had been troubled with indigestion since her menopause four years earlier. She had finally come into hospital because she had noticed a lump in her abdomen. She had not noticed any loss of weight, and on examination appeared to be fairly well nourished, although a little anaemic looking. The lump she complained of was found to be a large irregular mass in the upper half of her abdomen. Various procedures were carried out in the hope of obtaining some help towards diagnosing the origin and nature of the tumour, but all with negative results. A radiographic examination following a bismuth meal failed to reveal any abnormality. Complement fixation tests were tried on her blood to see if there was any evidence of infection with syphilis, hydatid, or tubercle, but negative results were obtained in all. It is interesting to note in the light of what was found at operation that a test meal revealed no occult blood, but the free hydrochlorie acid present corresponded to 27 c.c., and the total acidity to 52 c.c. of N/10 caustic soda. A laparotomy revealed

the abdominal cavity to be full of one matted mass of carcinomatous tissue. It was thought that the original growth was in the stomach, and from thence secondary growths had spread and involved the liver, omentum etc.

The values obtained for the serum proteins in these cases are remarkable. It must be confessed that before making the tests, the writer inclined to the view that normal results would be found. The low figures for the total amount of proteins in the last two cases, where the growths were more extensive, and the general effects on the patients greater than in the first, are worthy of note. It will have to remain for the future, when many more analyses such as the foregoing have been made, to show whether these results are fallacious, or represent the true state of affairs in malignant diseases. If they do, there appear to be two possible explanations. The view that carcinoma is an infection, although it has had its advocates, seems no longer tenable when the mass of the evidence accumulated against it is considered. (See reports of the Imperial Cancer Research Fund, 1905, [redacted]). But it may be that the view that the rise in the amount of globulins in infections is due to the infection itself will have to be revised and the idea, already put forward by some workers, that the phenomenon is due to the alteration in the permeability of the body cells be entertained. Or, it may be possible, since certain workers have shown that a certain degree of immunity can be produced against cancer by injection of the cancer cells (107), that it will be shown in the future that the body reacts to the presence of a malignant growth in somewhat the same way that it does to an infection.

In some of the cases, blood counts were performed at the same time approximately as the estimations of serum proteins. The results obtained may be contrasted in the following table:-

| Case No. | White Count. | Red Count. | Haemoglobin Per Cent. | Globulin Per Cent. | Remarks |
|---|---|---|---|---|---|
| 1.(Pernicious Anaemia) | 6,250 | 3,000,000 | 60 | 41 | |
| 2. " | - | - | 61 | 29 | |
| " " | - | - | 62 | 28 | |
| 3 " | 5,000 | 1,620,000 | 37 | 18 | |
| 4 " | 3,000 | 2,300,000 | 57 | 34 | |
| 5 " | - | - | 36 | 22 | |
| 7.(Bonti's) | 5,200 | 5,500,000 | 76 | 40 | |
| 8.(Lymphat. Leukaemia) | 256,500 | 2,500,000 | 54 | 56 | |
| " " | 215,000 | - | 55 | 13 | (6 wks. later, after benzol) |
| " " | 137,000 | - | - | 47 | (1 wk. later) |
| 9. " | 116,000 | 4,800,000 | 77 | 12 | |
| 10. " | 3,000 | 1,200,000 | 22 | 39 | |
| 11. " | 33,100 | - | - | 38 | |
| " " | 19,000 | - | - | 46 | (1 wk. later after benzol and x rays) |
| 20.(Nephritis) | 15,600 | 6,700,000 | - | 42 | |
| 25.(Auricular Fibrillation) | - | 3,000,000 | 60 | 11 | |
| 29.(Hodgkin's) | 12,000 | 5,000,000 | 91 | 37 | |
| 33.(Pyelitis) | 10,900 | - | - | 59 | |

It will be recollected that, at different times, the theory has been put forward that the increase in the amount of globulin depends, partly, at any rate on the accompanying leucocytosis, but that Hurwitz and Meyer (80) have shown that in infections the leucocyte count and globulin increases do not run parallel to one another, whilst Schmidt (82) has shown that the white count may be reduced with benzol without reducing the amount of globulin. The figures above provide additional confirmation of the view that there is no relation between the cell count and the percentage of globulin.

We wish to place on record here a peculiar fact noted in regard to the blood of the leukaemic cases. Usually on precipitating the proteins from the serum by acetic acid for the purpose of estimating the non protein constituents, it was only necessary to centrifugalize the solution for a few minutes at a low speed in order to separate the precipitate. The fluid thus obtained was crystal clear. In four out

of the seven examinations made in leukaemic cases, however, after such a procedure, it was found that the precipitate had not settled. Several experiments were tried on the same serum to see if it was merely an error in technique, but always with the same result. It was thought at first that the phenomenon was due to the proteins not being wholly precipitated, but it was found that this was not the case. The precipitate was found to be in such a fine, light, state, that it required spinning at high speeds for some considerable time to settle it. It was also noted in these cases that, if in repeating the experiment, a little stronger acetic acid was used, the usual form of precipitation took place. This led one to wonder whether in these cases the hydrogenion concentration of the blood might not be abnormal. In one patient (Case No. 8, examination 3) thanks to the kindness of a colleague, such a determination was made, but it was found that it lay within normal limits, the pH being 7.4. Unfortunately, at the time, no apparatus was available with which to determine the "alkali reserve" of the blood. In this case also (Case No. 8), there was a remarkably high percentage of non protein constituents, the readings for the three dates of examination being 2.4, 1.8 and 1.8 per cent respectively. An attempt was made to find the cause for this, and estimations of the blood urea, blood creatin, and creatinine, were carried out. But all these substances were only present in normal amounts, so that evidently some other factor was accounting for the high non protein percentages obtained. Later in the work, when the serum from cases of carcinoma was examined, a similar phenomenon happened in two of these analyses, (Cases No. 13 and 14) but it occurred in no other examination, so that whatever may be the change in the blood that produces this phenomena, it appears to be common to the serum of leukaemic and carcinomatous cases.

2. The Proteins in the Blood of Newly born Infants.

In the review of the literature given at the commencement of this thesis, it was seen that analyses of the blood of newly born animals revealed a low value for the amount of total protein, the decrease being mainly in the globulin fraction. It was shown that the amount gradually increased with age, reaching a constant adult value in a variable period depending on the nature of the animal under investigation. Further, Howe (97) has produced evidence to show that in calves,

there is an immediate increase in the globulin fraction after the taking of colostrum. In the course of the present investigation, the opportunity offered of examining the serum of four cases in which the blood had been obtained from the umbilical cord at birth for the purpose of performing the Wassermann test. In all the cases the specimen was taken from the placental end of the cord immediately after it was severed, but in the second case, in addition a sample was obtained from the child's umbilicus. The results were as follows:-

| Case No. | Non Protein Per cent. | Albumin Per cent. | Globulin Per cent. | Total Protein Per Cent. | Albumin expressed as Percentages of Total Protein. | Globulin |
|---|---|---|---|---|---|---|
| 15. | 1.2 | 3.6 | 3.1 | 6.7 | 54 | 46 |
| 16. | 0.9 | 1.9 | 4.1 | 6.0 | 32 | 68 |
| " (Taken from child's umbilicus) | 0.9 | 2.7 | 3.8 | 6.5 | 42 | 58 |
| 17. | 1.1 | 1.8 | 4.1 | 5.9 | 30 | 70 |
| 18. | 1.9 | 3.7 | 2.3 | 6.0 | 62 | 38 |

In all cases the mothers were young healthy women, who showed no trace nor gave any history of disease. The labours were all normal, and the babies healthy. The Wassermann test gave a negative reaction in each case. At the time that the tests were done, we knew of no cases in which the blood of newly born infants had been examined of recent years, so that the tests were carried out to see if human beings resembled other animals in the small amount of serum globulin present at birth. Afterwards the work of Lewis and Wells (98) was discovered, and, as was stated before, these workers found a similar condition of things to obtain in infants as Howe did in calves. We only desire to place the results obtained above on record here, and offer no comment on them at this time until further investigations have been concluded, for the specimens for examinations were not obtained by the author, and fallacies may have crept in through the mode in which they were obtained.

3. The Proteins in a Group of Renal and Cardiac Cases.

Six cases of nephritis were investigated, some of them rather exhaustively as can be seen from a reference to the case records in Appendix A. The results of the examinations were as follows:-

| Case No. | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent. | Total Protein Per Cent. | Albumin expressed as Percentages of Total Protein | Globulin |
|---|---|---|---|---|---|---|
| 19.(Chronic Interstitial nephritis during an exacerbation) | 1.4 | 3.4 | 2.7 | 6.1 | 65 | 45 |
| 20.(Chronic Nephritis due to lead) | 2.0 | 3.7 | 2.7 | 6.4 | 58 | 42 |
| " (3 days later) | 1.9 | 4.5 | 2.2 | 6.7 | 67 | 33 |
| 21.(Acute Nehpritis in child) | 1.4 | 1.8 | 4.1 | 5.9 | 30 | 70 |
| 22.(Acute Nephritis in boy) | 1.3 | 4.0 | 3.6 | 7.6 | 53 | 47 |
| 23.(Acute Nephritis in boy) | 1.2 | 5.2 | 2.7 | 7.9 | 66 | 34 |
| " (6 days later) | 1.8 | 4.4 | 2.8 | 7.2 | 61 | 39 |
| " (3 wks. later) | 1.6 | 4.9 | 3.0 | 7.9 | 62 | 38 |
| 24.(Acute Nephritis in man) | 1.5 | 3.9 | 2.0 | 5.9 | 66 | 34 |
| " (6 days later) | 1.8 | 3.3 | 1.8 | 5.6 | 68 | 32 |

The first case of the series was that of a comparatively young man, aged 29, who came into hospital complaining of dimness of vision, which on examination was found to be due to oedema of the retina, with typical "cotton wool patches," but no haemorrhages. His arteries were thickened and tortuous, whilst his blood pressure was, systolic 250, diastolic 180. The heart was not dilated. The urine showed the presence of heavy albumin and blood. The urea content of his blood was normal, and he gave a negative Wassermann test. There was no oedema present. It will be seen that the total amount of protein is low, whilst the globulin-albumin ratio is increased. In the second case (No. 20), a lead nephritis, the two analyses show that the total proteins, although on the low side, are just within the lower limit of normal values. The globulin-albumin ratio shows a small increase. The slightness of this variation, however, was surprising when it was considered in relation to the general condition of the patient, which was extremely bad. The man was drowsy, breathing stertorously, with heavy albuminuria. The systolic blood pressure was 210, the diastolic 130 m.m. of mercury. His blood revealed a retention of urea, the urea nitrogen being 75 m.g. per 100 c.c. The urinary diastatic index at 38 degrees C for 30 minutes was only one unit. The Wassermann test was negative. The slight change in the percentages of

globulin in this case will be referred to again later, in connection with some experimental work carried out.

The next two cases (Cases 21 and 22) were young males with acute attacks of nephritis which quickly subsided under treatment. An uneventful recovery was recorded in each case. Each of the patients revealed an increase in the percentage of globulin present, but it was much the greater in the former of the two, which was also the more severe clinically, with unconsciousness and fits at the commencement. The fifth case of the series was another acute nephritis in a boy, but here the tests were all carried out during convalescence and reveal little alteration. The last case of the series was that of an unfortunate man who was admitted to hospital suffering from lobar pneumonia. During convalescence, he developed a quinsy, and was just recovering from this when an attack of nephritis supervened. This last complication was not very severe for although there was heavy albuminuria at first, there was never any diminution in the quantity of urine, and the amount of urea in the blood was not raised. The serum proteins reveal a lowering in the total amount, but strange to say, no alteration in the globulin-albumin ratio.

As has been stated in the history, much attention has been paid to the estimation of the serum proteins in nephritis. Reiss used the estimation of the total proteins as a guide to treatment. Epstein (62, 63) pays particular attention to the protein values in this disease. He believes that the globulin-albumin ratio is not altered in the chronic interstitial - azotaemic - type, but only in the chronic parenchymatous - hydraemic - type. Nephritis also claimed Rowe's attention (91), and this writer made a special study of the chronic forms, finding that the globulin percentage was always raised except in uraemic persons just before death, thus disagreeing with Epstein's findings concerning the chronic interstitial type. Neither Rowe nor Epstein record much about acute cases. Rowe has published the results of his analyses in two such patients, but in each the nephritis was only a complication of another infection. From the results of the present investigation in acute cases, it would seem that the globulin-albumin ratio is altered according to the severity of the case, a mild attack having no effect. This agrees with Rowe's findings in infections as a whole, and with

Hurwitz and Meyer's work (80).

The values found in the serum from the patient with chronic interstitial nephritis (Case No. 19) support Rowe's findings against Epstein's contention that there is no rise in globulin in this condition. The case of chronic nephritis from lead poisoning remains for consideration (Case No. 20). It will be recollected that in this patient, in spite of the severity of the condition, there was very little change in the globulin percentage. Rowe examined one case of lead poisoning and found normal values for the serum proteins. These results were interesting for the following reason. It was thought that if in a case of nephritis the rise in the percentage of globulin was due to the infection, then in a nephritis due to chemical irritation as from lead, there should be no such alteration. As our analysis revealed that such might possibly be the case, an attempt was made to prove this experimentally. For this purpose, two healthy rabbits of approximately the same weight were taken, placed in "metabolism" cages, and their blood proteins estimated. The urine of both of them was found on examination to reveal nothing abnormal. Twenty four hours later, one of them received an injection of a solution of arsenic intravenously, the dose being 0.01 grams. of Arsenic per kilogram of body weight. The other rabbit was kept as a control, and each time blood was withdrawn from its mate, a similar quantity was taken from it also, and both specimens were analysed. The "metabolism" cages enabled the total quantity of urine passed to be collected and analysed.An hour and a half after the injection, the rabbit appeared to be in a state of irritation, moving continuously, and scratching itself. Its muscles were twitching continuously. Six cubic centimetres of blood were withdrawn for analysis. Three hours later it was in the same condition. No urine had been passed since the injection. An hour afterwards, its condition was unchanged, but it had passed seventy c.c's of normal urine. The next morning at 10 a.m., i.e., 13 hours after the injection, its condition was still unchanged, but it had passed another seventy c.c's of urine during the night. This specimen contained a faint trace of albumin, too little to estimate, and microscopically, many blood cells. Two hours later, another specimen of blood was taken. During the succeeding four hours it rapidly became worse. Its heart began to beat irregularly, and involuntary muscular twitchings came on. Finally the animal became comatose. No urine had been passed all day. At this

stage it was so bad that 10 c.c's of blood were withdrawn from the heart, and the rabbit killed. On a post mortem examination being performed, the heart was found to be dilated, the kidneys rather swollen, and the bladder empty. The Pathological Department made sections of the kidneys and reported that they showed the presence of an acute nephritis, in which the main damage was to the glomeruli. The serum protein examinations revealed the following:-

Control Rabbit.

| Date & Urine | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent. | Total Protein Per Cent. | Albumin expressed as Percentages of Total Protein. | Globulin |
|---|---|---|---|---|---|---|
| 19-3-23., noon | 1.6 | 3.9 | 1.5 | 5.4 | 72 | 28 |
| 20-3-23, 5 p.m. | 1.5 | 3.6 | 1.8 | 5.4 | 67 | 33 |
| 21-3-23, noon | 1.4 | 3.6 | 1.8 | 5.5 | 67 | 33 |
| 21-3-23, 4 p.m. | 1.5 | 4.0 | 1.9 | 5.9 | 67 | 32 |
| Rabbit which Received Injection. | | | | | | |
| 19-3-23, noon | 1.8 | 3.9 | 1.9 | 5.8 | 68 | 32 |
| 20-3-23, 5 p.m. (1½ hrs. after injection) | 1.5 | 3.9 | 2.2 | 6.1 | 64 | 36 |
| 21-3-23, noon (20 hrs. after injection) | 1.8 | 2.8 | 2.6 | 5.4 | 52 | 48 |
| 21-3-23, 4 p.m. (Rabbit comatose) | 1.7 | 3.5 | 2.2 | 5.7 | 61 | 39 |

These figures show only a very slight rise in globulin after the injection, so slight that it may have been accidental, especially as the next to last test which shows the high percentage (48%) of globulin was a bad estimation, owing to the difficulty of obtaining the blood, and the laking of the serum. Although the figures obtained from the lead nephritis case, and from this experimeht are not conclusive, yet they are suggestive. Unfortunately, lack of time prevented us from treating a number of rabbits similarly, and no further case of lead nephritis was available. However, this has opened up a line of research which might go far towards solving the problem of the rise in globulins. For it would appear from these results that in an attackof nephritis caused by an infective agent, the globulin-albumin ratio shows a pronounced rise. In a similar case, however, where the causal agent in a chemical irritant, no such alteration in the relative proportions

of the serum proteins occur. This would lead at once to the inference that the rise in the percentage of globulins is due to the bacterial invasion itself.

The question of the relation of the presence of oedema to the total amount of protein in the serum may now be considered. The results obtained in the present series have been tabulated, and are as given herewith.

| Case No. | Diagnosis and Remarks. | Total Protein Per Cent. |
|---|---|---|
| 19. | Chronic Interstitial Nephritis. Oedema had not been present, nor did it occur whilst the patient was under observation. | 6.1 |
| 20. | Nephritis due to lead. No oedema present, nor did it develop. | 6.4 |
| " | Three days later. | 6.7 |
| 21. | Acute nephritis in child. Had been very oedematous, but this cleared up four days before test and did not return. | 5.9 |
| 22. | Acute nephritis in a boy. Slight oedema present at time of test; had disappeared in a week. | 7.6 |
| 23. | Acute nephritis in a boy. Had been very oedematous, but this had cleared up a fortnight before the test. | 7.9 |
| " | Six days later. No oedema present. Condition improving. | 7.2 |
| " | Four weeks later. Practically well again. | 7.9 |
| 24. | Acute nephritis in a man following pneumonia and quinsy. Slight oedema present. | 5.9 |
| " | Six days later. Oedema disappeared. | 5.6 |
| 25. | Cardiac failure in pregnancy. Had oedema when walking about, but had disappeared now patient was in bed. | 7.1 |

It will be seen from these figures that the total proteins are, in all except one case, low in the presence of oedema, and may remain so for some time after its disappearance. Case twenty two, however, shows a normal amount when oedema was present clinically, and it is very hard to explain this, if Reiss' assumption is true, namely, that the lowering of the total amount of proteins [redacted] in these cases is due to a dilution of the blood which inevitably precedes and accompanies the oedema. For in that case, with oedema there must of necessity be found a decreased amount of serum protein. If other cases are found similar to this, it will be necessary to account for the decrease when

it occurs in some other manner. Unfortunately none of the cases in whom oedema was absent when the test was first carried out, developed it afterwards. So that no evidence has been gained either way for the view that the coming of oedema may be foretold by the appearance of a low value for the total proteins.

In many of the cases reported, estimations of the amount of urea present in the blood were made at the same time as the determinations of the serum proteins. The following table contrasts the values of the blood urea nitrogen and of the non protein constituents of the blood.

| Case No. | Diagnosis and Remarks | Blood Urea Nitrogen. | Non Protein Per Cent. |
|---|---|---|---|
| 7. | Banti's disease. Blood sugar estimated at same time was 0.09%. | 16 m.g. | 1.5 |
| 8. | Lymphatic leukaemia. Blood creatin and creatinine estimated at same time, and were normal. | 17 m.g. | 1.8 |
| 19. | Chronic Interstitial Nephritis B.P., $\frac{250}{180}$. | Normal | 1.4 |
| 20. | Nephritis due to lead. Patient drowsy, with stertorous breathing. Blood sugar at same time was 0.12%. B.P., $\frac{210}{130}$. | 75 m.g. | 2.0 |
| 21. | Acute nephritis; test done when pat. had been under treatment in hospital for 5 days and was recovering. B.P., $\frac{170}{120}$. | 12 m.g. | 1.4 |
| 22. | Acute nephritis. Test done about a week after onset. | 15 m.g. | 1.3 |
| 23. | Acute nephritis. Test done about three weeks after onset. B.P., $\frac{120}{80}$. | 15 m.g. | 1.2 |
| " | 6 days later. Patient improving and diet increased. Blood sugar 0.11% D $\frac{38^{\circ}}{30'}$ = under 4 units. | 18 m.g. | 1.8 |
| " | 4 weeks later. Practically recovered. | normal | 1.6 |
| 24. | Acute nephritis. Three days after onset. D $\frac{38^{\circ}}{30'}$ - 6.6. Urinary chlorides 1.03%. B.P., $\frac{150}{110}$. | 15 m.g. | 1.5 |
| " | 6 days later. | 16 m.g. | 1.8 |
| 25. | Cardiac failure in pregnancy. Heavy albuminuria present. D $\frac{38^{\circ}}{30'}$ +++ 20. B.P., $\frac{110}{70}$. | 11 m.g. | 1.8 |
| 28. | Retinites of unknown origin. No trace of any kidney disease. B.p., $\frac{175}{130}$. | 13 m.g. | 1.8 |

| Case No. | Diagnosis and Remarks. | Blood Urea Nitrogen. | Non Protein Per Cent. |
|---|---|---|---|
| 33. | Pyelitis B.P., $\frac{150}{110}$. | 22 m.g. | 1.4 |
| 34. | Rheumatoid Arthritis. Blood sugar at same time 0.09%. (Normal blood urea N = 11 - 25 m.g. Normal Non Protein % 1.1 - 1.3. Percentage error 0.1%) | 19 m.g. | 1.3 |

It was thought that it would be profitable to see whether the estimation of the non protein constituents would serve the purposes for which the blood urea test is now employed. For its performance apart from the estimation of the serum proteins, is much simpler and quicker than a determination of the blood urea, uric acid or creatin. It is recognised that in kidney inefficiency, the urea is one of the last substances to be retained in the blood, and that this detracts from the value of the blood urea test. Hence some workers have employed estimations of the uric acid or creatin and creatinine of the blood instead, as when the kidney is failing to function properly, a rise is shown in the amount of these, before it is in that of the blood urea. However, the tests employed in determining the quantity of any one of these three substances in the blood are much more accurate than the determination of the non protein constituents by the refractometric method. The former tests, well performed, have a percentage error of 0.001, whilst that for the latter is 0.1. As it was doubtful whether the non protein estimation would be delicate enough to show the retention of these bodies, except in large amounts. Of course, in its favour, is that it is estimating, not one of the non protein bodies, but all.

From the table just given it can be seen that in the one case recorded where the blood urea was raised in amount, the non protein percentage is correspondingly increased. But further, in several instances, the non protein estimation shows a marked rise, whilst the value for the blood urea remains normal. Case Number 23 is interesting in this respect, in that it shows a marked retention of substances in the blood according to the non protein estimation, when the diet was increased, but the amount of blood urea shows no rise. That there was some such retention is borne out by the urinary diastatic index which was below four units. Again in Case 24, the blood urea estimation was normal on each occasion. This was only to be expected, for the patient was

receiving practically no protein food. But the urinary diastatic index was low, and the non protein percentage high, showing that there was some kidney inefficiency. From these examples, it would appear that the non protein estimation is a very satisfactory test to employ. On the other hand, it must be admitted that it sometimes shows an increased value in cases where at present our knowledge prevents us attaching any significance to it. Thus in Case No. 8, no cause could be found to account for the high non protein percentage. In case No. 25, where there was heavy albuminuria accompanying a failing heart, and examination revealed no trace of any nephritis, the non protein percentage was 1.8. It is interesting to note in this case the way the kidney was "leaking," as shown by the urinary diastatic index of +++ 20. Again, in Case Number 23, where, owing to the existence of retinitis, the presence of nephritis was suspected, but could not be proved by any of the ordinary clinical means, the non protein percentage was raised. Does this mean that the high non protein content was the earliest biochemical evidence of a nephritis concealed clinically except in its causation of a retinitis; or was the high non-protein content in this case due to some other cause than nephritis? So although the state of our knowledge will not permit of this test being used alone as yet to the replacement of blood urea, uric acid, or creatin and creatinine estimations, by its use in conjunction with these and other similar tests useful additional information may be obtained, and eventually a further insight gained into the excretory function of the kidney.

Before leaving this table, it should be pointed out that in several cases, the blood sugar was determined along with the other two estimations. It has been reported that this is often raised in quantity in patients suffering with nephritis, but no such phenomenon was formed in any case of this series.

In concluding this series of cases, four others may be profitably considered, as they afford a contrast to those given above. The first (Case No. 25) is that of a young married woman aged 24, whose heart failed during pregnancy. She became so ill that eventually labour was induced and a still born eight months old foetus was delivered. She recovered slightly after this but was admitted to hospital two

months later in great distress, vomiting continuously, with a weak irregular pulse and oedema of the ankles. There was heavy albuminuria. Examination revealed no trace of any nephritis. The blood proteins in this case were as follows;-

Non protein 1.8%

Albumin 6.3%

Globulin 0.8%

Total proteins 7.1% of which the globulins formed 11%.

The oedema at the time of the test had completely disappeared. It will be seen that there was no lowering of the total proteins, and no rise in the globulin-albumin ratio. The next case was that of a married man of 58 who complained of shortness of breath and epistaxis. His arteries were thickened and tortuous, and his blood pressure greatly raised (Systolic 200, Diastolic 110). The lungs and heart were healthy, however, and he was passing a good quantity of urine, with a specific gravity ranging from 1012 - 1025. Repeated examinations of the urine failed to reveal any abnormality. The blood urea was determined twice and each time showed a normal value. The Wassermann test was negative. The blood proteins were as follows:-

Non Protein 1.6

Albumin 4.2

Globulin 3.1

Total proteins 7.3 of which globulin formed 42%.

The third case was that of a woman 60 years old, who was very breathless and weak. Her heart was irregular with a "to and fro" murmur heard all over the precordium, being at its maximum over the aortic area. There was also an area of dulness in the second and third intercostal spaces, and a pulsation in the jugular notch. It was thought probable that an aneurysm was present. The Wassermann test was negative. An estmation of the serum proteins was performed to see if this would show any evidence of an infection. The result, however, was quite normal, being

Non protein 1.4%

Albumin 7.0%

Globulin 1.7%

Total proteins 8.7 of which globulin forms 20%.

A radiographic examination later failed to show any aneurysm.

The last case of the four is that of a married woman aged 56, who went to an oculist because of failing vision. The oculist found a retinitis to be present, and sent her to a physician for examination. She was found to be passing a fair quantity of urine with a good range of concentration. Examination of it showed no abnormality. The blood urea was normal. The blood proteins were

Non protein 1.8%

Albumin 6.3%

Globulin 1.4%

Total proteins 7.7 of which globulin forms 19%.

On this evidence, it was judged that there was probably no nephritis present. Six months later, she was still in quite good health, so that up to the present the favourable prognosis given seems to have been justified.

4. The Proteins in a Group of Infections.

Blood was obtained at different times from patients suffering with different conditions due to an infecting agent. The results found in the various conditions were as follows:-

| Case No. | Non Protein Per Cent. | Albumin Per Cent. | Globulin Per Cent | Total Protein Per Cent. | Albumin expressed as percentages of Total protein | Globulin |
|---|---|---|---|---|---|---|
| 29. Hodgkin's Disease | 1.5 | 3.9 | 3.0 | 6.9 | 56 | 44 |
| 30. Lobar Pneumonia | 1.9 | 3.6 | 3.9 | 6.5 | 40 | 60 |
| 31. " " | 1.4 | 3.8 | 2.4 | 6.2 | 61 | 39 |
| 32. Rheumatic Fever | 1.6 | 1.2 | 5.1 | 6.3 | 19 | 81 |
| 33. Pyelitis | 1.4 | 2.9 | 4.2 | 7.1 | 41 | 59 |
| 34. Rheumatoid Arthritis | 1.3 | 2.7 | 3.8 | 6.5 | 42 | 58 |
| 35. Typhoid Fever | 1.3 | 2.2 | 4.4 | 6.4 | 34 | 66 |
| 36 " " | 1.1 | 2.9 | 2.5 | 5.4 | 54 | 46 |

These results are just what one would expect to find, the total proteins being lowered in the acute stages of the disease, and the globulin per centage showing a marked increase. No recent analyses of the serum proteins have been published, as far as could be gathered, in cases of Hodgkin's disease, Rheumatic Fever or Rheumatoid Arthritis, but the values obtained here fall into line with those obtained in other infections.

The case of rheumatoid arthritis was interesting in that a most rigorous examination failed to reveal any forms of infection. The marked rise in globulin, however, suggested that such was the cause, and since then the patient has shown marked improvement following injections of a Bacilli Coli Communis Vaccine intravenously. Attention should be drawn also to the results in the two cases of Typhoid Fever. Rowe has published the results of an estimation in one such case, finding that the globulin was not increased in amount. In both these patients, there was a marked rise in the globulin-albumin ratio. Bearing in mind the fact that different workers have found that if, in producing immunity to Typhoid, large doses of vaccine, causing a reaction are given, there is an increase in the globulin percentage, but that with carefully controlled doses, no such rise is experienced, an explanation of the differences in the results may be offered. Possibly it may be found in future cases that where there is a marked reaction on the part of the body to the invasion by the bacillus typhosus, and the case is of comparatively short duration, a rise in globulins will occur. But where there is but little reaction, resulting in a long drawn out case, no alteration in the globulin-albumin ratio will be found. If this proves to be the case, determinations of the serum proteins in typhoid fever will be of some prognostic value. So that an interesting field for further research opens up in this direction, and much interest will attach to future determinations of the serum proteins in typhoid, accompanied by a study of the condition of the patient and the duration of the disease.

## Summary.

In the following summary, the advances in medical knowledge made in the present investigations are indicated.

1. An historical summary of the work done on the subject of the serum proteins up to the end of the year 1922 has been provided, with a full bibliography. This was badly needed, especially in the English language. Previous reviews have been published in German, but none later than 1914, and the only account in English which is at all full is that by Rowe. It is seven years since this was published, and it contains no account of the numerous researches carried out by the refractometric method.

2. Values for the serum proteins have been obtained in cases of lymphatic leukaemia, carcinoma, Hodgkin's disease, rheumatic fever, and rheumatoid arthritis. No estimations, performed by modern methods, have previously been published in the case of these diseases.

3. Confirmatory results have been obtained for the values obtained in various other diseases by Rowe.

4. A detailed study of the variations in the serum proteins in pernicious anaemia, leukaemia, and nephritis have been given, a full clinical survey of the case having been made in conjunction with the analysis of the proteins in each case.

5. Analyses of the serum proteins in the blood of newly born infants have been given. The results obtained suggest that further confirmation is required before Howe's results for calves, obtained by a different technique, are accepted for infants.

6. The occurrence and origin of the rise in the globulin-albumin ratio in disease have been fully discussed. Evidence has been brought forward to show that loss of albumin through the kidneys cannot explain the increase of globulin in the blood in disease, and confirmation has been given to Schmidt's work that the alteration in the white count has no relation to this phenomenon.

7. The result of the investigation has shown several directions in which research may now be directed with profitable results; namely, by making further estimations in cases of carcinoma, mineral nephritis, and typhoid fever.

8. The present clinical applications of the test, namely, in nephritis and syphilis have been pointed out. In addition, two additional uses have been put forward. It has been shown that value attaches to the estimation of the non protein constituents by the refractometric method. Also, it is suggested, the phenomenon of the rise in the globulin-albumin ratio may be made use of in diagnosing the infectious origin of obscure conditions.

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# APPENDIX A.

## Records of Cases Quoted in Text.

CASE NO.1. DISEASE:- Pernicious Anaemia.

Name:- Mr W.D. Age 47 M. Occ.:- Engine Driver.

2.8.22 Patient first got ill six months ago, when he complained of stiffness in legs, and feelings of pins and needles, and difficulty in walking, lost appetite, had feelings of nausea with retching. He had been losing weight and become short of breath. Pat. came into hospital and on examination was found to have a pernicious anaemia with a subacute combined degeneration. Improved with arsenic and was discharged after two months. Readmitted four months later, i.e. two weeks ago, with same symptoms aggravated.

O.E. Temp. normal P.80 reg. fair vol. and tension. Arteries. Respiration 20.
Tongue Eyes Lungs Heart
Abdomen:- Liver and spleen palpable.
Nervous system: sensation not impaired. Tendon jerks exag:
Power in arms and legs:- Incoordination present. Ataxia and
Rhombergism present. Ankle clonus both sides.
Urine 1012 acid No albumin or sugar.
Blood: Reds 3.000.0000 Whites 6.250 Hb.60% Col.Ind.1
2.8.22 Film showed typical pernicious picture.
Blood proteins: Non proteins 1.7% Albumens 3.7%
Globulins 2.6%
Total proteins 6.3% of which 59% is albumin and 41% globulin.

28.8.22 Hb. went up to 76% and he was sent home.

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CASE NO. 2. DISEASE:- Pernicious Anaemia.

Name :- Mr J.C. Age 61 M. Occ.:- Machinist.

7.2.23 Patient was admitted into hospital three months ago complaining of increasing weakness with shortness of breath for three months preceding. Has had no swelling of legs.
Appetite poor, no indigestion. Bowels open regularly: no diarrhoea
Urine and micturition normal.

P.H. NO previous illness, always been healthy.
S.H. Non smoker: moderate drinker (beer)
V.H. Negative.
F.H. Good.
O.E. On admission patient showed no signs.
Temp. was normal. Pulse. Respiration.
Eyes. Teeth. Throat.
Heart. Lungs. Abdomen.
Reflexes. Sensation. Urine.
Blood: Hb. 42% Red cells 2.100.000. Col.Ind.1.05
Film showed picture of pernicious anaemia.
Patient was put on Arsenic and improved so that when he left the hospital six weeks ago, his Hb. was 71%. Then he caught a cold and Hb. went down to 61%. Is better again now and is improving. Looks well and putting on weight.

1.2.23 Blood protsins ~~0.2%~~ ~~of albumin~~ albumin 71% and globubin 29%.
(good test) Non protein 1.6% Albumin 4.4% Globulin 1.8%.

8.2.23 Patient improving. Hb. 62%.
Blood proteins . Non protein 1.3% Albumins 4.6% Glob. 1.8%
Total proteins 6.4% of which Albumins 72% and Globulins 28%.

APPENDIX A'

CASE NO..3 DISEASE:-Pernicious Anaemia.

Name:-Mrs FM. Age 48. M Occ..House wife.

25.10.22 Patient gaveahistory of havigg an attack of diarrhoea eight years ago.At that time was living under insanitary conditions with no deep drainage and several in house had diarrhoea . Patient was in hospital then for eight weeks.She recovered slightly,but has remained with frequent loose motions ever since.For the last six mnoths has become weaker,and lately appetite has gone .Now has 4-8 loose brownish motions early in morning,after which is all right for the rest of the day. Has normal formed motion at night.Has never passed any blood.

P.H. Scarlet fever and typhoid when 7 years old .Before onset eight years ago was quite healthy.

M.H. Menopause 3 years ago.Nodischarge since.

O.H. Has one child aged 24,healthy.Has had 3 miscarriages after this: 4,4,and 8 mnnths respectively.Was torn badly at confinement and had 3 operations torepair this afterwards.

F.H. Good,

O.E. Pale,lemon colored women.
Pupils,equal,and react to light and accomodation. Fundi.
Tongue:glazed, smooth andatrophic.
Heart. ✓ Lungs. ✓ Abdomen. ✓ Reflexes. ✓
Urine. ✓
Various examinations were then carried out .
On admission blood count was : Red cells 2,000,000.. White cel 5,000. Hb.39%. Col. Ind. .85.
Pathologist reported the picture was suggestive of a pernicious anaemia.

Gastric test meal revealedno blood,but absence of HCl and presence of lactic acid.

Bismuth meal showed nothing abnormal in stomach,duodenum or colon.

A recent blood count gave Red cells 1,620,000. White cells 5,000. Hb. 37%. Col.Ind.1.0.
Film showed anisocytosis and poikilocytosis.

Stools revealed no blood,but ova of parasites and no B.dysenteriae in culture. Wassermann was negative.

23.11.22. Non protein 1.4% Albumin 5.4%. Globulin 1.2%.
Total proteins 6.6%,of which albumin 82%,and globulin 18%
This was not a very successful experiment,however,as there was trouble with the Amm.sulph..

7.12.22 Patient much the same .Only having 3 or 4 motions first thing in morning,and then is all right for rest of day.

Film showed pernicious anaemia.
Blood proteins:- Non proteins 1%. Albumin 4.0%. Globulim2.2
Total proteins 6.2% of which albumin was 65%. and globulin36%
This was a very good determination ,enough serum being obtaine to do two tests and so conform the results.

APPENDIX A.

CASE NO..4. DISEASE:-Pernicious Anaemia.

Name:- Mr C.T. Age 45 M. Occ.. Gardener.

6.2.23 Patient complained of pains in stomach and sensation of pins and needles in hands .He commenced to get this pain 15 months ago,but for some time before that had felt tired and languid. Pains bear no relation to meals.

At the same time,patient had a senation of pins and needle in toes.This has extended up to his knees,has started in his fingers.Now his feet feel numb,and he is not able to walk well

Appetite is fair.No vomiting.Bowels constipated.No diarrhoea Urine and micturition normal.Suffers frequently from headaches

P'H. Typhoid and pneumonia when 12 years old. Was in hospital 12 months ago for same complaint .

Old notes of that time give a similar history and a diagnosis of pernicious anaemia.Two test meals were done ,both of which showed no HCl,free lactic acid [redacted] occult blood.The faeces were examined on 3 separate occasions and no blood was found. A bismuth meal was done but X ray showed no abnormality.

The blood picture was however definitely a pernicious anaemia,with Hb 81%,and Col.Ind.1.1. Red cells 2,200,000. White cells 2,500. Film showed megalocytosis and gave typical picture.

F.H. Good.

S.H. Non smoker and non drinker.

V.H. Negative.

6.2.22.

O.E. Temp.normal. Respiration.✓ Pulse reg.good vol.and tension.

Eyes: pupils equal,and react to light and accomodation.

Tongue: clean.

Lungs. ✓ Heart. ✓ Abdomen.✓ Reflexes ✓

Sensation: normal.

Urine: pale,clear. 1020. Acid. No albumin or sugar.

Blood:- Redcells 2,300,000. White cells.3,000. Hb.67%. Col.Ind.1.3.

Film typical of pernicious anaemia.

Blood proteins. Non proteins 1.4%. Albumin 4.5%. Globulin2.3 %. Total proteins 6.8%,of which albumins 66% and glob.34%*

8.2.22 Patient ran away from hospital,and went home.

APPENDIX A.

CASE NO..5. DISEASE:- Pernicious Anaemia.

Name:-Mr A.L.. Age 69. W. Occ:-Stone cutter.

2.8.22 Patient was admitted six months ago complaining of weakness which was increasing and was becoming very short of breath. He gave a past history of having had rheumatic fever 35years ago :also bronchitis and measles.Was here a month ago with colitis.

O.E. No physical signs were detected ;but blood gave typical picture of pernicious anaemia.
Red cells 1,780,000: White cells 5,000. Hb.28%.
Patient was put on arsenic for two months and improved.Hb. went up to 40%,and put on weight.
Since then has remained much the same. Hb. now is 36%.

2.8.22 Blood proteins. Non proteins 1-1%, Albumins 5-0%. Glob.1.4%. Total proteins 6.4% of which albumin is 78% and globulin 22%.

3.8.22 Patient has been gradually getting worse .Hb.is now down to 13%.He stays in bed all the time.Spleen palpable:also liver. Red count 1,200,000. Whites 10,000.

12.9.22 Patient died.

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CASE NO..6. DISEASE:- Secondary Anaemia.

Name:- Mr W.M.. Age 46 M. Occ.. Labourer.

2.8.22. Patient was admitted five months ago ,complaining of weakness of the legs ,feelings of giddiness ,and a tendency to fall over.
Does not suffer with headaches. Bowels open regularly. Micturition normal. Appetite poor. Memory failing during the past twelve months.

P.H. Pleurisy twenty years ago. Went blind in left eye years ago for six months:but recovered. In hospital year for some trouble

V.H. Gonorrhoea 13 years ago.

S.H. Moderate smoker.Heavy drinker.

O.E. On admission: Temp.97. Pulse 100 Regular:fair vol.and tension. Blood press. 126/72 Resp.24. Arteries. ✓
Eyes. ✓ Teeth: bad with pyorrhoea.
Heart. ✓ Lungs: Rhonchi all over. Abdomen. ✓
Reflexes:- No tendon jerks elicited. Planter flexor response not elicited. Rhombergism present.Walks with a wide base. No incoordination. No loss of sense of position. Both legs up to knee painful on press
Urine: 1008. Alk., no albumin or sugar.
Sputum: no T.B..
Cerebral spinal fluid. ✓
Blood count:- Red cells 4,700,000. White 11,200. Hb.47%.
Film showed no abnormality. Differential: polymorphs 76%. lymphocytes 24%, eosinophils 1%.
Put on Fe.and As. remained much the same. became
Then 2 months later, ~~developed~~ plantar reflex extensor on both sides.Wasting of leg muscles.
Wasserman test on blood and cerebral spinal fluid negative.
Then a month ago on blood count being done again,reds were 5,200.000. whites 15,000. Hb.58%. Col.IND.0.88.
Film showed a secondary anaemia with an eosinophilia.
Faeces were examined for parasites.None were detected.
Legs were then wasted,spastic and flexed.
Blood proteins: Non proteins 1.3%. Albumin 2.0%.Globulin4.0%.
Total proteins 6.0% of which 33% is albumin and 66% globulin.

APPENDIX A!

CASE NO..7. DISEASE:.Banti's Disease.

Name:-Mrs G.T.. Age 46. M.. Occ: House wife.

30.1.23. Patient was admitted into hospital 14 months ago complaining of attacks of choking sensations with shortness of breath. Attacks came on at night and lasted for a month or so. Gave a history then of having had a cough for two years, with loss of voice every now and then during that time. Much worse before admission with much phlegm. Also gave a history of losing weigh in last 9 months, having night sweats, and becoming out breath on least exertion.

O.E. On admission. Patient was found to have a few rhonchi at right apex posteriorly, but eyes, nose, throat and mouth were normal. Specialist could detect nothing to account for loss of voice. Edge of liver was palpable. Patient was in hospital then for 5 months, during which time she had several haematemeses.
Sputum was examined for T.B., but was negative.
Wasserman was negative.
X ray showed changes at the hilum of lung and at the apices, which suggested chronic bronchitis, which might be due to T.B..
She developed a friction rub in left infraclavicular region for a time. Later had teeth removed. Finally was discharged in much the same condition as when admitted. Had no temp. all the time.
Was readmitted 9 months later (1.12.22), with a history of attacks of abdominal pain since she left hospital, accompanied by vomiting. Pain bears no relation to food: is in right upper abdomen. General weakness increasing.

P.H. Patient had two operations, 2½ and 6½ years ago but the cause was unknown.

F.H. One brother has T.B.: five children healthy: two miscarriages.

M.H. No periods for four months, before that regular.

O.E. Temp. normal. Pulse. ✓ Respiration. ✓
Eyes. ✓ Voice husky. Tongue. ✓ Teeth false.
Heart. ✓ Lungs. ✓
Abdomen:- spleen and liver both palpable: firm and smooth.
Reflexes. ✓ P.V.:- nothing abnormal detected.
Has several attacks of epistaxis since admission.
Blood count was normal. Red cells 3,800,000. White 6,200.
Hb. 67%. Differiential was normal.
Faeces gave a slight positive test for occult blood.
Blood sugar 0.09%. Blood urea 16 m.g. per 100 cc.
Wasserman done again and was negative.

17.1.23 Blood proteins:- Non protein 1.5% . Albumin 4.6%. Globulin 3.1%.
Total proteins, of which 60% are albumin, and 40% globulin.

30.1.23. Blood proteins:- Nonproteins 1.2%. Albumin 3.7%. Globulin 3.7%
Total proteins 7.4% of which 50% are albumin and 50% globulin.
Patient is in statu quo. Urine has no albumin, sugar or other abnormalities. Liver and spleen still enlarged. Skin is certainly becoming pigmented.
Diagnosis of Addisons discussed. Also malingering sugested.
The Honorary thought that with the enlarged spleen and liver and attacks of perisplenitis, the case was certainly one of Banti's disease, though an atypical one. The case was also seen by the surgeon with a view to splenectomy, but he advised against it.

APPENDIX A.

CASE NO..8. DISEASE:-Lymphatic Leukaemia.

Name:-Mr J.L.. Age 68. M. Occ..Labourer.

5.2.23 For past 12 years has been feeling he could not do quite as much work. Two years ago he began to feel really knocked out and had treatment for 2 months at an institution for "hot water cure".which did him no good.
Since then has been becoming successfvely weaker and weaker.
Has lost a lot of weight and become very pale.
Appetite has failed.No indigestion.Bowels constipated.
Urine and micturition normal.
Has no vomiting or headaches.
Patient first noticed lumps in neck 12 years ago,but took no notice of them.Used to become more prominent with cold.
Two years ago he noticed lumps in armpits and groins which have increased as much as those in neck. Have never been tender or caused him any pain.Finally become so weak that a fortnight ago he collapsed and has been in bed ever since.

P.H. Had enteric in W.A. 12 years ago.Had growth removed from buttocks at same time.Frequently had attacks of quinsy 50 years ago,but for past 40 years has been quite free of it.
Has never had tonsils removed.Does not suffer with sore throat

F.H. Good.

V.H. Negative.

S.H. Open air work. Non smoker. Moderate drinker.

5.2.23

O.E. Temp.99.2. Pulse 100 reg.. Respiration 28.
Pupils. Tongue:-furred. Teeth,all false.
Throat:-tonsils enlarged.
Lungs. Heart.
Abdomen:- liver enlarged to 3" below costal margin.
spleen enlarged and palpable:both firm and reg.
Reflexes.
Lymphatic glands:- enlarged in neck,axilla and groin. Firm, not tender on pressure.Discreet,not matted or fixed.
Urine:- 1015. Acid. No albumin or sugar.

7.2.23 Gland from neck incised. Histological picture was that of a lymphatic hyperplasia.
Blood picture:- Red cells 2,500,000. White 256,500.
Hb. 54%.
Differential count: small lymphocytes 80%,
large lymphocytes 18%. Neutrophil polymorphs 1.0%
Myelocytes 1%*
The film showed the picture of a lymphatic leukaemia. The majority of the cells are lymphocytes the majority of which are embryonic in type.
An occasional myelocytew and polymorph are seen.
Blood proteins:- Non ptotein 2.4%. Albumins 2.1%. Glob.2.7%*
Total proteins 4.8% of which albumins are 44%, and globulin are 56%. This was a very good test except that proteins did not ppt.properly with acetic acid.
Wasserman test was negative.
Patient was put on Benzol 0.3. t.d.s.

23.2.23- Been on benzol all the time. Red cells 3,600,000. White cells 302,000. Hb. 64%.

8.3.23. Benzol increased to 0.9 t.i.d.

20.3.23. White blood count 215,000. Hb. 55%*

23.3.23. Blood proteins:- Non proteins 1.8%. Albumins 5.4%.
Globulins 0.8%.
Total proteins 6.2%,of which albumin equals 87% and globulins 13%. (Very good test with unlaked serum: but proteins did not ppt.properly with acetic acid.)

25.3.23. Benzol increased to 1.2 t-i-d.

27.3.23. White blood count ,187,000. Is feeling better and has been getting up. Spleen is slightly smaller again now,although it showed a slight increase than first put on Benzol.
Blood proteins:- Nonprotains 1.8%. Albumins 3.4%. Glob. 3.3%.

APPENDIX A'.

CASE NO ...3 continued.

Total proteins 6.7% of which albumins are 53% and globulins 47%
(Very good test with unlaked serum. Once again the proteins were not ppt'd. properly by the acetic acid.)
Blood urea:- 17 m.g. per 100 cc. pH. equalled 7.43.

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CASE NO..9. DISEASE:-Lymphatic Leukaemia.

Name:- Mr H.H.. Age 55. M. Occ..Commercial traveller.

25.2.23. Patient has been ill for about a year, and under treatment. When first seen, he had enlarged spleen and liver, massive abdominal glands and large glands in groins, axillae, less in neck.
White blood count was over 200,000. and blood gave the picture of a lymphatic leukaemia.
There was no sign of any infection to be discovered.
Patient was put on Benzol and improved. White cells diminished and 3 months ago was nearly normal, 10,000, when Benzol was discontinued
At present patient feels well: glands are just the same.
Is doing his work and says he is in quite good health.
Blood count was:- Red cells 4,800,000. White cells 116,000.
Col. Ind. 0.8. Hb. 77%.
Film, a few nucleated reds were present.
Differential red count:- Polymorphs 2.7%, lymphocytes 97%.
Basophils 0.3%.
Wassermen test was negative.
Blood proteins:- Non proteins 1.3%. Albumins 6.8%. Glob. 0.3%
Total proteins 7.0% of which albumins are 88%, glob. 12%.
(Very good test with serum unlaked.)

---

CASE NO..10. DISEASE:- Lymphatic Leukaemia.

Name:-Mr H.A.. Age 66. W.. Occ..Labourer.

2.8.22 Patient was admitted 2 months ago with diarrhoea, which had been present for two months. During same time had noticed lump in left axilla.
P.H. Typhoid 35 years ago . Pleurisy 4 years ago.
V.H. Gonorrhoea 20_30 years ago.
F.H. Says he always has a "cold in the head". Had a stricture 16 years ago, which was dilated, and he has had no trouble since.
Has frequency D 3_4/N 2_3. NO pain. Sometimes loses control of urine.
O.E. On admission.
Temp. normal. Pulse 108 reg..Fair vol. and tension.
Arteries. ✓ Respiration 20.
Tongue: furred. Teeth: all false.
Heart. ✓ Lungs. ✓
Abdomen:- liver palpable, 2" below costal margin.
Glands:- of neck, axillae and inguinal regions all enlarged.
Urine:- Alk.. 1007. No albumin or sugar.
Blood count on admission. Red cells, 4,000,000.
White cells, 24,000. Hb. 53%. Col. IND. 0.6%.
Blood film: reds show changes in size and shape: and some immature forms. Differential count showed 87% of lymphocytes.
Was put on soamin and benzol.
Has remained much the same since; count now is; red cells 1,200,000 White 3.000. Hb. 22% Was a preponderance of lymphocytes

APPENDIX A.

CASE NO.. 10 continued.

2.8.22 Blood proteins. Non proteins 1.2%. Albumins 3.9%.Glob.2.5%. Total proteins 6.4% of which 61% is albumin, and 39% glob..

31.8.22 Gradually become weaker and finally died.

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CASE NO..11. DISEASE:-Acute Lymphatic Leukaemia.

Name: Mr W.H.K.. Age 29. M.. Occ..Station master.

7.2.23

Patient was admitted into hospital 6 weeks ago complaining of swelling under the arm and in groin.Has noticed them come several times in the last3years,but they have always subsided. With this attack,however,which commenced 5 weeks ago,they have gone on increasing in size.Patient is short of breath. Bowels constipated. Micturition normal.

P.H. No past illness.No history of sore throats,nose trouble,or any chronic infection.

F.H. Good.

V.H. Negative.

S.H. Non smoker: non drinker.

O.E. On admission:-

Temp. was normal. Pulse. ✓ Respiration. ✓

Eyes. ✓ Teeth: all false. Throat. ✓

Lungs. ✓ Heart. ✓

Abdomen:- liver enlarged: edge palpable. Spleen also enlarged and palpable.

Glands:- enlargement of lymph glands in neck,both axillae and both inguinal regions. Are not tender: discreet.

Blood:- Red cells 5,000,000. White cells 41,000. Hb. 92%.

Some-Red cells showed some anisocytosis,and polychromasia Nucleated forms were present. 89% of the white cells are immature lymphocytes.

Patient was put on Arsenic and Benzol,on which the glands slightly diminished in size and the white cells decreased, so that on 24.2.23, white cell count was only 10,300. Benzol was then stopped,and cout went up again so that on 1.2.23 it was 33,100.

1.2.23 Blood proteins:- Non proteins 1.8%. Albumins 4.2%. Glob.2.6%. Total proteins 6.8%,of which albumins are 62% and globulins are 38%. (Test was a good one.)

8.2.23 Back on Benzol again. Blood count then was white cells 15,000 Stared X ray applications to glands 1 week ago.Feeling very well and is back at work.

Blood proteins:- Nonproteins 2.0%. Albumins 3.6%. Glob. 3.1%. Total proteins 6.7%,of which albumins are54%,and globulins are 46%.

14.2.23 Has been still having benzol and X rays. Hb. 57%. Red cells 3,200000. White cells 2,500. Col.Ind. 0.9.

APPENDIX A.

CASE NO .. 12. DISEASE:-Epithelioma of Tongue.

Name: Mr E.W.. Age 68. M.. Occ.. Labourer.

29.3.23. Patient was admitted a fortnight ago, complaining of swelling of both sides of the neck. Causes great difficulty in swallowing and in mastication.
Appetite good. Bowels open regularly.
P.H. Good.
V.H. Negative.
S.H. Used to be a heavy drinker and smoker.
O.E. Well nourished man.
Temp. normal. Pulse 80. reg.. Good vol. and tension.
Eyes. ✓ Mouth:- epithelioma on right side of tongue. No ulceration.
Glands:- enlarged on both sides of neck.
Heart. ✓ Lungs. ✓ Abdomen. ✓
Reflexes. ✓ Urine. ✓
Was considered inoperable.
Wasserman was negative.
27.3.23. Blood proteins:- Non proteins 1.1%. Albumins 3.7%. Glob. 3.4
Total protein 7.1%, of which albumin is 52%, and globulin is 48%.
(Good test with unlaked serum.)

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CASE NO..13. DISEASE:-Carcinoma, Gastric.

Name. Mr G.S.. Age 63. S. Occ..Invalid pensioner.

29.3.23 Patient was admitted into hospital 10.3.23, complaining of pain in stomach which came on 10 minutes after meals.
Been ill for 4 months. Pain often makes him vomit, and this relieves the pain. Has vomited bright blood 2 or 3 times in the 4 months.
Bowels have been loose for last 2 months. Never any tarry motions
Micturition:- has no pain or difficulty, but has to pass it once in the night.
Has lost 11 lbs. in the last 9 months.
Has had cough for past 25 years and brings up a lot of yellow phlegm.
P.H. Suffered with bronchitis, and asthma for years, which has kept him unhealthy.
S.H. Moderate drinker and smoker.
V.H. Negative.
O.E. On admission, Temp. was normal. Pulse 76. Resp. 20.
Patient thin with sallow tint to skin.
Eyes. ✓ Tongue. ✓ Teeth. ✓ Neck. ✓
Lungs:- emphysematous. Heart. ✓
Abdomen:- hard, indefinite mass in epigastrum.
No glands in groin. Legs. ✓ Urine. ✓
11.3.23. Test meal done: showed deformity of greater and lesser curvature; diagosed as carcinoma without stenosis of pylorous
U.E. Midline incision: large carcinoma found involving whole circumference of fundal portion of stomach. Glands in hilum of liver. Left untouched and abdomen closed.
28.3.23. Wound healthy. Patient quite well. Has had no temp. since operation or signs of infection.
Blood proteins:- Non proteins 1.4%. Albumins 1.1%. Glob. 3.9%. Total proteins 5.0% of which albumins are 22%, and globulins 78%.
(Good test, except that proteins did not ppt. properly with acetic acid. Serum unlaked.)

APPENDIX A.

CASE NO..14. DISEASE:- Carcinoma.

Name:-Miss M.W.. Age 47. S. Occ..Nurse.

28.3.23. Patient was admitted into hospital on 12.3.23,complaining of a "lump in the abdomen and indigestion."
Quite well until 4 years ago,when she began to suffer with indigestion;full feeling after food with flatulence.Felt sick but did not vomit.Went on like this until 7 months agowhen had "gastric influenza".Ill for about 4 weeks,with increase of all symptoms given above.Since then has not picked up.
Has not noticed any loss of weight.
Bowels constipated.No history of tarry motions.
Urine and micturition normal. No vomiting.

P.H. Scarlet fever when a child.No hydatid history.Clots in leg 10 years ago.

M.H. Menopause at onset of digetive symptoms.Before this had been quite regular. No diarrhoeaat any time.

O.E. On admission.
Temp.normal. Pulse reg.. Respiration. ✓
Eyes. ✓ Tongue.✓ Teeth : false.
Neck:-no glands. Heart. ✓ Lungs.✓
Abdomen:-irreg.tumor in upper half of abdomen. Movesfrom side to side. Reaches to umbilicus. Apparently is not continuous with liver. Gives no thrill.
Legs. ✓ Reflexes. ✓ Urine.✓

13.3.23. Test meal done: revealed- free HCl.27. Total acility 52.
No occult blood.
Blood count. White cells 14,000. Differential count:
Polymorphs 88%. Eosinophils 1%. Lymphocytes 11%.
Bismuth meal: revealed no abnormality of stomach or duodenum.
Blood was sent Melbourne for Wasserman,and Hydatid and Tubercle complement fixation test. All were negative.

28.3.23. Patient in statu quo. To have operation.
Blood proteins:- Non proteins 1.7%. Albumins 2.0%. Glob.3.7%.
Total proteins:5.7% of which albumins are 35%,and globulins 65%.
(Good test except that proteins did not opt. properly).

29.3.23. Operation revealed carcimoma:probably gastric in origin,but now with deposits in liver,and omentum. Abdomen was one mass of secondary growths.

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CASE NO..15. [redacted].- Placental Blood.

Name..Miss A.. Age 17. S.. Occ.. Home duties.

Labour normal,10p.m.(25.1.23) - 2p.m.(26.1.23).
Placenta -complete. Membranes- ragged.

30.1.23. Patient was quite normal and healthy; nothing detected on examation.
Pregnancy normal. Baby healthy.
Wasserman test negative.
Blood prteins:- Non proteins 1.2%. Albumins 3.6%. Glob. 3.1%.
Total proteins 6.7%,of which albumins are 54%,and globulins 46%.
(Test :-a very good one.)

APPENDIX A.

CASE NO..16. Placental Blood.

Name: Mrs B.. Age 2[illegible] M.. Occ..Home duties.

Pregnancy: third.
Labour: normal. One suture. Placenta and membranes complete.
Labour: midnight - 4.[illegible]a.m..
Patient was quite healthy and has always been so.
Pregnancy normal. Baby healthy.
In this case blood was obtained at deliyery from cord, both from the placental end and from the baby's umbilicus.
Results were:-

| | Placental end. | Child. |
|---|---|---|
| Non protein | 0.9% | 0.9% |
| Albumin. | 1.9% | 2.7% |
| Globulin | 4.1% | 3.8% |
| Total protein | 6.0% | 6.5% |
| of which albumin | 32% | 42% |
| globulin | 68% | 58% |

(Test were both good ones).

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CASE NO..17. Placental Blood.

Name: Mrs F. Age 23. M. Occ.. House-wife.

Labour:normal. 3p.m. - 10p.m.. Placenta:complete.
Membranes:ragged.
Patient was quite healthy and examination revealed nothing.
Gave no past history of illnesses.
Pregnancy normal. Child healthy.
Wasserman negative.
Blood proteins:- blood from placental end of cord.
Non protein 1.1%. Albumins 1.8%. Glob. 4.1%.
Total proteins 5.9%, of which 30% are albumin and 70% g
globulin.
(Test was a good one).

---

CASE NO..18. Placental Blood.

Name: Mrs S. Age 19. M. Occ..House-wife.

17.1.23. Labour- Forceps, under chloroform. Membranes- ragged and piece retained.
Patient was quite healthy:so also was baby. Gave no history of past illnesses. Always enjoyed good health.
Examination revealed nothing abnormal.
Pregnancy was quite normal.
Wasserman negative.
Blood proteins:- Blood was taken from umbilicalcord at delivery, from placental end.
Non proteins 1.9%. Albumins 3.7%. Glob. 2.2%.
Total proteins 6.0%, of which albumins are 62% and glob. are 38%.

APPENDIX A.

CASE NO..19. DISEASE: Chronic Nephritis.

Name:MrR.L.. Age 29. S..... Occ..Saddler.

19.9.22. Patient has not been feeling well for the past 3 months: was suffering with shocking pain up back. Then a fortnight ago eyesight became blurred,and has remained the same since. Has had to give up work. Has had no treatment outside. Appetite fair:does not suffer with indigestion.Bowels open regularly every day.
Urine and micturition normal:but for last fortnight has had to get up once at night.
Used to suffer with bad headaches: with feelings of nausea about 9 months ago,but lately has not been troubled with them. Has had a slight cough lately. Thinks she has lost weight.
P.H. Measles when young. Away at the front for 3 years. No illnesses. Left leg amputed. Always been healthy.
V.H. Negative.
S.H. Smoker (pipe). Moderate drinker(wine).
O.E. Healthy looking man.
19.9.22. Temp.normal. Pulse92. regular. Large Vol.and tension
Respiration 20. Arteries thickened and tortous.B.P. 250/150
Pupils react to light and accomadation. No paralysis.
Retina shows oedema and cotton wool patches but no haemorrhage
Tongue: furred. Lungs. ✓ Heart:not dilated, no bruit. Abdomen. ✓ Legs. ✓R. ✓.
Left leg amputated. Reflexes. ✓
Urine. 1012, heavy albumin,no sugar. Blood present.
25.9.22. Blood proteins- Non proteins 1.4%. Albumin 3.4%. Glob.2.7%. Total proteins 6.1%,of which albumins are 55% and glob.45%.
Blood urea normal.
Wasserman negative.

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CASE NO..20. DISEASE: Nephritis.

Name..Mr C.W.. Age 55. M.. Occ..Plumber.

24.7.22 Patient was admitted complaining of pain across back of neck; general weakness and attacks of paresis and numbness of the extremities.
Pain at back of neck been present on and off for past 12 month Spasm lasts a week. Has had 3 attacks of paresis assoc. with feeling of numbness. First one was 19 months ago on right side. Onset sudden, lasted for about ½ hr.,but did not recover fully for 8 weeks. Second attack was left side,8 months ago: third was 8 weeks ago. This last attack was less severe.
Patient thought the cause of these attacks was a shock he had 30 years ago at work.
Appetite good: suffers with indigestion. Bowels constipated, takes salts.
Micturition normal;no frequency. Sleeps well.
P.H. Leaded 18 years ago; from trade. Mainly colic. Left leg broken 16 years ago.
V.H. Negative.
S.H. Heavy smoker, till present illness. Moderate drinker.
F.H. Good.
O.E. Patient very drowsy with stertorous breathing. Talks with a slurred speech.
Temp.normal since admission. Pulse 96,good Vol.and tension.
Arteries thickened. B.P. 250/150.
Face: no paralysis. Eyes;react to light and accomadation.
No nystagmus; no paralysis.; maked proptosis of left eye.
Tongue: thick fur;not tremulous,projected in mid line.
No blue line.
Lungs. ✓ Heart: not dilated,mitral symbolic murmur.
Abdomen. Liver. ✓ N. AD..
Legs; peculiar symetrical,bluish staining of skin over both knees. Old fracture visible. Wasting of muscles of left le

APPENDIX A..

CASE NO..20 continued.

Reflexes: Tendon jerks all exaggerated. Plantar flexor.
Sensation: Heat,cold,touch;joint sensation unimpaired.
No incoordination. No rhombergism.
Walks unsteadily,and drags left leg.
Urine: 1015.Acid. Heavy albumin,no sugar. Many casts:few epithelial: mostly granular.
In private,patient very hysterical. Since he has been in hospital has been very emotional. Since admission has been on light diet with no meat.
Having Pot.Iod.,Mag Sulph.,and Pot.Cit..

24.7.22. Blood count: Red cells, 6.700.000. White cells, 15.800. Film shows no abnormality.
Albumin 0.35%. Venesected 2 days ago; 10ozs. withdrawn.
B.P. fell to 184/98, rose again to 210/130, today.

Blood urea N.equals 75 mg.per 100 cc..
Blood sugar equals 0.12%.
Blood proteins: Non proteins 2.0%. Albumin 3.7%. Glob. 2.2%. Total proteins are 6.4%,of which 58% is albumin and 42% globulin.

27.7.22. Blood proteins: Non proteins 1.9%., Albumin 4.5%., Glob. 2.2%. Total proteins 6.7%,of which 67% is albumin,and 33% globulin.

Urinary Diastase under 1 unit.
Urea concentration: 1st.hour 1.2%; 2nd. hour 1%. (Maclean's test.)
Wasserman negative.

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CASE NO..21. DISEASE: Acute Nephritis.

Name: Mr D.K.M.. Age 8. S.. Occ.. School boy.

5.10.22. Patient was admitted and gave history of having been ill for two weeks before admission. Illness commenced with swelling of neck and face. Then 3 days before admission ~~became drowsy~~, had abdominal pain and vomiting;also severe headache. Finally on day of admission became drowsy,and finally lost consciousness and had 7 fits . No history of sore throat.

O.E. Patien was unconscious;face oedematous. Tongue dry and brown
Heart. ✓ Lungs. ✓ Abdomen. ✓
Urine 1018. Acid. Heavy albumin, no sugar or blood.
B.P. 170/120. Microscop., urine contained blood cells and epithelial casts.

10.10.22. Five days later,oedema had disappeared and felt quite well.
12.10.22 Blood urea N. equals 12m.g. per 100 cc.
13.10.22 Blood proteins: Non proteins 1.4%. Albumins 1.8%. Glob. 4.1%. Total proteins 5.9%, of which albumins are 30%. and globulins 70%.
21.10.22 B.P. 120 (systolic)
Urine contains no albumin now. Patient sent home.

APPENDIX A.

CASE NO..22. DISEASE: Acute Nephritis.

Name:Mr A.M. Age 10½. S.. Occ..School boy.

Patient was admitted into hospital complaining of swelling of privates,which came on suddenly 2 days before admission. No pain accompaning enlargement.
For the 5 or 6 days before this,the patient had complained of pain in the abdomen,which bore no relation to food. Has lost appetite. No vomiting.

P.H. Measles 3 years ago. Sore throat 6 weeks ago. Has had impetigo lately. Has large sores on knees at present.

F.H. Good.

O.E. Face puffy and oedematous.

7.9.22. Temp.normal. Pulse 90 reg.. Respiration 24.
Pupils: react to light and accomodation. Teeth.✓ Tongue.✓
Lungs.✓ Heart.✓ Abdomen.✓
Scrotum oedematous.
Legs: slight oedema of both ankles.
Reflexes.✓
Urine: 1030. Acid. Heavy albumin,no blood or sugar.
Microscopically:- granular casts,and blood present.
Albumin 1.5%.

12.9.22. Blood urea 15m.g.per 100cc.. Albumin ⅛% cleared up.
Urine urea 2%.

13.9.22. Blood proteins: Non proteins 1.3%. Albumin 4.0%.Glob. 3.6%.
Total protein 7.6%,of which albumin is 53%,and globulin 47%.

16.9.22. Oedema only very slight now. Urine contains very slight alb..

24.9.22. Discharged quite well. Been up for 3 days.
No oedema. Been passing a normal amount of normal urine.

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CASE NO..23. DISEASE: Acute Nephritis.

Name: Mr E.C.. Age 14. S.. Occ..School boy.

15.6.22. Patient was bumped at football a month ago,and developed large lump on right thigh.Came into hospital with it ,and was in hospital for a week. Then went home;lump in leg swelled up again,and in a few days face also became swollen.
He saw a doctor who said he had "nephritis",and sent him to hospital. At this time felt quite well,and had no pain.
Noticed nothing wrong with water. Before bump,had been quite well. Urine and micturition had been normal. Bowels opened regularly. Did not suffer with headaches,except slight ones about once a month.
No trouble with eyes. No cough. Was gaining weight before illness.

P.H. Measles when 4 years old.
Diptheria when 10 years old.

F.H. About 2 years ago used to suffer with sore throats about once a month,from tonsils. Been all right lately.
Has father,mother and sister who are quite well; 3 brothers who all died of some "swelling up"disease like dropsy,when children.

O.E. Healthy looking lad. Temp. 96. Respiration 18.
Pulse 72: big Vol.,small Tension Regular. B.P.,
Eyes: pupils react to light and accomodation.
Tongue.✓ Teeth.✓ Tonsils:enlarged.
Heart.✓ Lungs.✓ Abdomen: fluid in both flanks.
Legs: slight oedema of ankles. Thigh quite better.
Reflexes: knee jerks active and equal. Plantar flexor.
Urine: on admission continued heavy albumin+blood.
S.G. 1034. Acid.
Quantity at first averaged 13ozs per diem: now averaging 40ozs per diem.
On 7.6.22. had 2.3grms. alb. per litre.
On 9.6.22. had .75grms. alb. per litre.

APPENDIX A..

CASE NO..23. continued.

27.6.22. Blood urea N. equals 15grms per 100cc. No oedema, feels well.
Blood proteins: Non protein 1.2%. Albumin 5.2%. Glob. 2.7%.
Total proteins 7.9%,of which albumin is 66%,and glob.34%.
3.7.22. Feels well,no oedema. Eating ravenously. B.P. 120/80.
Passed 55ozs.of urine in last 24 hours. Albumin 1.5%.
Blood proteins: Non protein 1.8%.Albumin 4.4%. Glob.2.8%.
Total protein 7.2%,of which albumin is 61%,and glob.39%.
Blood urea is 18 m.g. per 100cc.
Blood sugar 0.11% 33/30' equals below 4 units.
9.7.22. Still very well. Averaging 40 ozs. of urine for past week.
17.7.22. Albumin 0.1%. Blood urea 15m.g.per 100cc.
Albumin equals .075%. Averaging 50 ozs. per unit a day.
31.7.22. On light diet with no meat.
Blood urea normal.
Blood proteins: Non protein 1.6%. Albumin 4.9%. Glob.3.0%.
Total protein 7.9%,of which albumin is 62%.and glob. 38%.

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CASE NO..24. DISEASE: Acute Nephritis.

Name: Mr R.G.. Age 29. M.. Occ..Painter.

28.6.22. Patient was admitted on 21.5.22. suffering with pneumonia.
Had no urinary symptoms then,and his urine contained no albumi
or sugar. Had a lobar pneumonia at right base.
Heart then was not dilated. Temp.dropped to normal on 28.6.22
by crisis,and was convalescing when on 11.6.22,i.e.3 weeks
after admission developed sore throat,which went onto tonsilla
abscess,first on left,then on right side. These got better,
and was convalesent again,when on 26.6.22. complained of head-
ache,and had a convulsive fit in which he became cyanosed and
unconscious.
26.6.22. O.E. Temp. 97.2. Pulse 102. reg.. Respiration 26.
Heart: A.B. 5th space; 10½cm. from mid line.
C.D. Right border of sternum to third space to apex.
No bruits. B.P. 155/110.
Lungs,no oedema.
Slight oedema of ankles,and slight lumbar pad.
Urine: 1022. Acid. Heavy albumin and blood.
Microscop: blood casts, leucocytes and phosphates.
Put on milk and water. To have Pulv.Jalap.Co. and vapour
baths.
28.6.22. Albumin 8%.
Temp. has been keeping normal. Pulse 80-90.
In last 24 hrs. has passed 32ozs. of urine.
29.6.22. Blood urea N equals 15grms. per 100cc.. Diastatic 146.6.
Albumin for past 24 hrs. is 0.43%. Passed 38ozs. of urine.
S.G. 1010. Alk. No blood.This morning only showed faint trace
of albumin. B.P. 144/92. NaCl. equals 1.03% in urine.
On the 27.6.22. d 38/30' equals 6.6.
3.7.22. Blood proteins: Non proteins 1.5%. Alb. 3.3%. Glob. 2.0%.
Total proteins 5.9%,of which 66% are alb. and 34% glob..
Urine since 27.6.22.

| | S.G. | Quantity. | Alb.%. | Total Albumin for 24hrs.in grms |
|---|---|---|---|---|
| 27.6.22. | 1020. | 36ozs. | .4%. | 4.3grms. |
| 28.6.22. | 1020. | 38ozs. | .3%. | 5.4grms. |
| 29.6.22. | 1000. | 29ozs. | .05%. | .45grms. |
| 30.6.22. | 1008. | 50ozs. | .05%. | .75grms. |
| 1.7.22. | .... | 100ozs. | .... | .... |
| 2.7.22. | 1008. | 70ozs. | .075%. | 1.6grms. |
| 3.7.22. | 1006. | 78ozs. | .... | .... |
| 4.7.22. | 1010. | 90ozs. | .15% | 4.95grms. |
| 5.7.22. | 1003. | 60ozs. | .... | .... |

APPENDIX A...

CASE NO..24.continued.

5.7.22. Blood urea N. equals .16m.g. per 100cc.
Blood proteins: Non proteins 1.8%. Albumin 3.8%. Glob.1.8%. Total protein 5.6%,of which 68% is albumin and32% glob.

12.7.22. B.P. 120/90. No oedema or headache. Very well.

Light diet. Well. B.P.125/85.

12.7.22. Urine:

| | S.G. | Quatity. | Alb.%. | Total alb. |
|---|---|---|---|---|
| 6.7.22. | 1008. | 62ozs. | .1%. | 1.8grms. |
| 7.7.22. | 1010. | 90ozs. | .. | .. |
| 8.7.22. | 1008. | 60ozs. | .. | .. |
| 9.7.22. | 1020. | 60ozs. | .1%. | 1.8grms. |
| 10.7.22. | 1020. | 56ozs. | .. | .. |
| 11.7.22. | 1020. | 40ozs. | .05%. | .6grms. |
| 12.7.22. | 1020. | 50ozs. | .. | .. |
| 13.7.22. | 1020. | 44ozs. | .1%. | 1.1grms. |
| 14.7.22. | 1020. | 56ozs. | .. | .9grms. |
| 15.7.22. | 1020. | 32ozs. | .1%. | .. |
| 16.7.22. | 1020. | 1400cc. | .. | .. |
| 17.7.22. | 1020. | 1200cc. | .. | .. |
| 18.7.22. | 1020. | 900cc. | .. | .. |
| 19.7.22. | 1010. | 1800cc. | .1%. | .. |

Blood urea N. equals 15m.g. per 100cc.

19.7.22. B.P. 120/80. Having fish,chicken.bread and butter etc. Very well.

26.7.22. Blood urea N. equals 16.6m.g. per 100cc. Still passing an average of 1500cc. a day. Albumin remains the same at .05%. Feels well.Gets up now. Diet increased b t not having much meat or eggs now.

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CASE NO..25. DISEASE: Auricular Fibrillation.

Name: Mrs R.G.. Age 24. M.. Occ.. House duties.

24.10.22. Patient came into hospital 3 months ago,complaining of dyspnoea,cough,and pain in back; said she had been like this for 12 weeks,during which time she had been in bed. Had occas. coughed up blood.
Was pregnant at time: thought she was 5 months. Had lost appetite,and suffered with vomiting. No pain in abdomen.
Bowels open regularly.
Had frequency for past 12 weeks. N 2-3,D 3-4.

M.H. Regular till 5 months ago,then ceased.

P.H. No rheumatic fever,tonsillitis,or sore throats.
Influenza 3 years ago,since when has never been well; suffers with heart trouble;knocks up on least exertion.
Previously had been very healthy.

F.H. Good.

On examination was dyspneic and cyanotic. Temp.normal. Pulse was 130 and irregular. Respiration 30-40. B.P.110/70. Heart was enlarged with presystolic and systolic murmurs. Lungs: signs of oedema at both bases. Abdomen: liver enlarged to 1" below C.M. Reflexes. ✓ Signs of pregnancy were present.
Urine was 1026. Acid. No albumin or sugar.
Was in hospital for a fortnight,on Digitalis,and did not improve. Finally transferred to gynaecologist,who induced labour,and a full term child,stillborn,was delivered 3 weeks after admission.
A week later had attack of vomiting,and urine was found to contain albumin. No casts found. No other examination was made apparently. After another week,felt better.Pulse was slow but irregular,albumin was down to a trace,sowas sent home and told to continue under a private doctor.

23.11.22. Readmitted into hospital. Complains that she started vomiting 24 hours agoand has been ever since; has pain in back and suffers with headache. Has frequency of micturition but no pain

APPENDIX A..

CASE NO..25.continued.

Notices legs swell up when she walks about.
Bowels regular. Has not lost weight.

O.E. On admission; patient pallid;in distress,vomiting continuously
Pulse irregular,weak . Temp. normal. Respiration. ✓
Lungs: ✓ Heart:enlarged. Presystoic and systolic not audible but has diastolic at base.
Abdomen. ✓ Legs. No oedema.
Urine: heavy albumin. Lab. reported pus cells and few casts, but did not say what kind. Numerous gram negative bacilli were present(not a catheter specimen).

24.10.22. Blood urea:N. 11m.g. per 100cc.
Blood proteins: Non proteins 1.8%. Albumin 6.3%. Glob. 0.8%. Total proteins 7.1,pf which albumin equals 89% and globulin 11%.
Diastatic index more than 20.
Blood count: Red cells 3,000,000. Hb.60%. Col.Ind.0.8%.
Albumin 0.02%.
Patient continued to have albumin in urine. Could not take the Digitalis as it made her vomit.
Died 20.11.22-,after being in a failing condition for a couple of days,with an unattainable pulse.

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CASE NO..26. DISEASE: Hyperpyesis.

Name: Mr A.M. Age 58. M.. Occ. Engine Fitter.

9.7.22. Patient complains of shortness of breath,and bleeding from nose. Says was quite well until 6 weeks ago;when began to notice shortness of breath,which after 3 weeks forced him to give up work. Then while resting at home began to have attacks of bleeding from the nose,two or three times a day,which gradually grew more severe. Bleeding was generally only of short duration. This has practically stopped since he has been in hospital.
Patient otherwise feels quite well.Appetite good. No indigesti
Bowels open regularly every day.
Urine and micturition normal. No headaches. No cough.
Thinks he has been gaining weight lately.

P.H. Influenza 17 years ago.Always been very healthy
S.H. Smoker:pipe, 1oz a week. Moderate drinker (beer).
V.H. Negative.
F.H. Good.
O.E. Healthy looking man.

9.7.22. Temp.been normal since admission. Pulse varies between 80-60
Regular, fair vol.and tension. Arteries: atheromatous and tortuous. B.P. 198/110.
Pupils:equal and react to light and accomodation.No nystagmus.
Mucous membranes rather anaemic.
Tongue:furred. Teeth: all false.
Lungs. ✓ Heart: cardiac dullness; mid sternum to third rib to 3½" from mid sternum in fifth intercostal space.
Sounds ✓ Abdomen. ✓ Legs. ✓ Reflexes. ✓
Urine: 10ozs. No albumin or sugar. Been on low diet.

9.7.22. Urine for past few days: 6.7.22. 16ozs.
7.7.22. 22ozs.
8.7.22. 34ozs.
S.G. on 8.7.22. varied between 1025 and 1012.
B.P.: 205/110.
Blood urea N. equals 15m.g. per 100cc.

11.7.22. Urea concentration test: First hour: 0.5% urea.
Sec. hour: 1.1% urea.
Fundi: arteries, arterio-sclerotic with indentation of veins
Otherwise ✓.

APPENDIX A..

CASE NO.. 26.continued.

17.7.22. Still having slight attacks of epistaxis. On milk diet.
B.P.. 150/100. Wasserman negative. No headache,feels well
Averaged 38ozs of urine a day for the past week.
Blood urea N.: 16.6m.g. per 100cc.
Urinary urea: 0.8% during same hour. therefore urea concentration factor equals 30.

18.7.22. Urea concentration test: First hour: 0.4%.
Sec. hour: 0.8%.
Urine.✓ X ray shows no aneurysm.

24.7.22. B.P.190/110Feels better.No more nose bleeding.
Is having about 1100 calories a day;with very little protein.
Diastatic index 6.6.

31.7.22. Phenolsulpholthalein test$ : First hour: 40%.
Sec. hour: 35%.
Blood proteins: Non proteins 1.6%. Alb. 4.2% Glob.3.1%.
Total protein 7.3%,of which 58% is alb. and 42% glob.

13.8.22. On diet. B.P. down to 180/90.
Sent home.

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CASE NO..27. DISEASE: Arterio Sclerosis.

Name: Mrs M.C.. Age 60. M.. Occ.. House duties.

18.9.22. This was a patient who came in complaining of breathlessness, andweakness.
She had an area of dullness in second and third right intercostal spaces. Heart was irregularand was to and fro murmur heard all over precordium,maximum intensity in aortic area.
Had pulsation in jugular notch and just below.
Was considered possible that she had a aneurysm.
B.P. 185/70. No tracheal tug.
Wasserman was negative,so thought possible that blood proteins would reveal evidence of an infection. These however, were normal
Blood proteins: Non proteins 1.4%. Alb. 7.0%. Glob. 1.7%.
Total proteins 8.7%,of which 80% is albumin and 20% glob
X ray later showed a dilated heart with a widening of the aortic arch,but no definite aneurysm.

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CASE NO..28. DISEASE: Suspected case of Nephritis.

Name: Mrs B. Age 56.. M.. Occ..House Duties.

18.10.22. Patient was sent from eye specialist with diagnosis ofiretinitis of unknown origin. Complained of dimness of vision,headache and indigestion. Had errors of refraction which he corrected and headaches now better.
No frequency. No other history.
P.H. Good.
V.H. Negative.
O.E. B.P. 175/100. Arteries thick and tortuous. Heart.✓
Lungs.✓ No oedema.
Urine:1025. Acid. No alb. or sugar. Microscopically nothing.
25.10.22. Blood ureaN.: 13m.g. per 100cc.
25.10.22. Blood proteins: Nom proteins 1.8%. Alb. 6.3%. Glob. 1.4%.
Total proteins 7.7%,of which alb. is 81% and glob. 19%.

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APPENDIX A..

CASE NO..29. DISEASE: Hodgkin's Disease.

Name: Mr J.E.. Age 49. S.. Occ.. Driver.

30.1.23. Patient was admitted one month ago, complaining of swelling in neck. Swellings commenced as small lumps twelve months ago, which have gradually increased in size until some of the masses are as big as a small orange. Other wise he feels quite well. Has no swelling elsewhere. No weakness or tiredness. Appetite good. Not lost weight . No night sweats.

P.H. No serious illness.

F.H. Good.

V.H. Negative.

O.E. Stout man with collar of glanduler swellings.
Temp. ✓ Pulse. ✓ Respiration. ✓
Pupils react to light and accomodation. Teeth. ✓ Tongue. ✓
Throat. ✓ No evidence of any septic focus.
Neck: enlarged glands on both sides. Glands firm and discreet; not tender. Not attached to skin or deep structures.
Heart. ✓ Lungs. ✓ Abdomen. ✓ Urine. ✓
No glands enlarged in axilla or groin.
A gland was removed and sections made. Report on sections was thath the gland showed a histological picture resembling that of Hodgkin's with marked infiltration by eosinophils and hyperplasia of endothelial cells. Throughout the gland are areas which appear to be definitely neoplastic. Probably the tumour represents a neoplastic(endothelioma) development on top of a Hodgkins reaction.
X ray picture showed enlargement of glands at hilus of lungs.
Blood count: Hb. 91%. Red cells 5,000,000. Col.Ind.0.9.
White cells 12,000. Film: no abnormality.
Differential shows 8% eosinphilia, polymorphs 78%.

23.1.23. Blood proteins: Non proteins 4.0%. Alb. 3.2%. Glob. 1.9%.
Total proteins 5.1%, of which 63% are alb. and 37% glob.
(Test a good one, but done on a very hot day.)

1.2.23. Has had no treatment since last week. In statu quo.
Blood proteins: Non protein 1.5%. Alb. 3.9%. Glob. 3.0%.
Total proteins 6.9%, of which 56% are alb. and 44% glob.
(Very good test).

---

CASE NO..30. DISEASE: Pneumonia.

Name: Mr W.P.. Age 50. S.. Occ..Driver.

Patient had a cold for 3 weeks before admission, which 3 days before became much worse; sputum became sticky and rusty, and felt feverish.

P.H. Lumbar pneumonia a year ago.

O.E. On admission: Temp. 102. Pulse 120. Respiration 50.
Appears flushed. Eyes. ✓ Tongue: moist and furred.
Heart. ✓ Lungs: signs of lobar pneumonia at right base
Abdomen ✓

2.8.22. Venesected. B.P. before venesection 120/70. After 130/80.
Cyanosis very much improved.
Wasserman positive.
Blood proteins: Non proteins 1.8%. Alb. 2.6%. Glob. 3.8%.
Total proteins 6.8%, of which alb. are 40% and glob. 60%.

5.8.22. Temp. has been down to normal but up again.
Venesection wound infected with pure pneumoccoci.

12.8.22. White count up to 30,000.
Temp. still going up and down. White count 15,000.
Aspirated got 12ozs pus. Smears showed pneumoccoci in great quantities.

---

# APPENDIX A.

CASE NO..31. DISEASE: Acute Lobar Pneumonia.

Name: Mr S.. S..

12.9.22. Patient came into hospital in a unconscious state.
Blood proteins: Non proteins 1.4%. Alb.3.8%. Glob. 2.4%.
Total protein 6.2%,of which 61% was alb. and 39% glob..
Patient died.

---

CASE NO..32. DISEASE: Rheumatic fever.

Name: Mr E.W.. Age 25. S.. Occ..Labourer.

Patient had rheumatic fever in 1914,and again 5 months ago.
Never properly recovered after last attack,and now has come in much worse again.

19.8.22.O.E. On admission:
Temp. 100,6. Pulse 96. Respiration 24.
Heart. ✓ Lungs. ✓ Abdomen. ✓
Knees both swollen and flushed,also left ankle and joints of fingers.
Urine: 1020. Acid. Slight alb.,no sugar or pus.
Was put on salicylates,and rapidly improved.
Blood proteins: non proteins 1.6%. Alb. 1.2%. Glob. 5.1%.
Total proteins 6.3%,of which 19% are alb. and 81% glob..

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CASE NO..33. DISEASE: Pyelitis.

Name: Mr C.W.. Age 41. M.. Occ.. Barman.

6.8.22. Patient has had attacks of renal colic on and off for 17 years about every 3 or 4 months. Attacks last from 4 hours to 4 days. Was brought in unconscious following one of these attacks and was said to have been having fits with vomiting.
Gives an history of severe headaches during the attacks,suffers with frequency of micturition.Says water is milky,but there is never any blood in it,to his knowledge.
The Dr.who attends him in private gave him a history of pyelitis with pus in the urine,for the last 12 years.

O.E. On admission: had a very flushed face with stertorous breathing
Temp. 102. Pulse regular. Arteries thickened.
B.P. 150/110. Eyes. ✓ Tongue: furred.
Teeth: poor. Heart. ✓ Lungs. ✓
Abdomen: slight tenderness over kidneys,but nothing palpable.
Reflexes. ✓ Urine: Acid. Contains blood,pus and albumin.
Put on Pot.Cit..

6.8.22. Was venesected,and 24ozs withdrawn.
Temp. went down to normal day after admission,and stayed down.
Urine: contained cell casts and B. Coli Communis.

7.8.22. Wasserman negative.
Blood urea N. equals 22m.g. per 100cc.
Blood proteins: Non protein 1.4%. Alb. 2.9%. Glob. 4.2%.
Total proteins 7.1%,of which 41% is albumin, and 59% glob.
White count: 10,940.

14.8.22. Improving. Temp. normal. Still much pus. B.P. 135/100?

---

APPENDIX A ..

CASE NO..34. DISEASE: Rheumatoid Arthritis.

Name: Mrs D.. Age 50. M.. Occ.. House duties.

27.3.23. Patient had menopause 18 months ago. Had had slight pain in ankles for years. About 6 months ago got pain and swellings in wrists, fingers and knees; and slightly in ankles which gradually became worse.

P.H. No previous illnesses; except cancer of the breast removed 5 years ago.

F.H. Good. Patient has had two children, both of which are healthy.

O.E. Teeth: all false. No tonsils. Lungs. ✓
Heart: aortic systolic, thought to be haemic and of no importance. Abdomen. ✓ Joints: typical rheumatoid arthritis.
Urine: normal. Catheter specimen revealed no infection.
~~The Sugar~~ No trace of any infection detectable.

27.3.23. Blood sugar 0-~~09~~%.
Blood urea 19.5m.g. per 100cc..
Blood proteins: Non protein 1.3%. Alb. 2.7%. Glob. 3.8%.
Total proteins 6.5%, of which 42% is alb. and 58% is glob..
(Good test; serum not laked ).

27.3.23. Has had a little irregular pyrexia in the evenings since has been in hospital.

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CASE NO..35. DISEASE: Typhoid Fever.

Name: Miss D.B.. Age 19. S.. Occ.. Housework.

Patient was taken ill on 6.2.23. with headache, vomiting and indefinite pains in legs.
Was admitted into hospital 6 days later in much the same state

P.H. Quinsy 12 months ago.

F.H. Good.

O.E. On admission:
Temp. 104. Pulse 144. regular. Respiration 20.
Pupils equal and react to L. + A.. Tongue very dirty with thick fur.
Lungs. ✓ Heart. ✓
Abdomen: few typhoid spots, spleen not palpable. Otherwise good.
Reflexes. ✓ Urine. ✓
Was found to have White count 4,000, and gave positive Widal to B. Typhosus, so confirming diagnosis.
After this ran a typical typhoid course for a month.
Temp. came down to normal 12.3.23. and stayed down to normal for a week. Urine was examined on 6 occasions. Always normal.
Blood proteins were taken during this period.

19.3.23. Non proteins 1.3%. Alb. 2.2%. Glob. 4.4%.
Total proteins 6.4%, of which 34% are alb. and 66% glob..
(Was done with a badly laked serum.)

2.4.23. Had a relapse on 21.3.23, and since then has gone through the whole course again, but temp. is down to normal again for first time today.

---

APPENDIX A.

CASE NO..36. DISEASE. Typhoid Fever.

Name. Mr C.W.A.. Age 16. S.. Occ..Librarian.

26.3.23. Patient had been ill for 6 days when admitted to hospital. There was vomiting and epistaxis at onset. Has lost all appetite.
P.H."Gastritis" 2 years ago. Chicken pox when infant.
O.E.On admission.
Temp.104. Pulse 104. Respiration 40.
Eyes.✓ Tongue: furred. Teeth.–
Lungs.✓ Heart.– Abdomen: few roseolar spots.
Spleen palpable.
Urine 1020, acid, no albumin or sugar or pus or blood.
27.3.23. White count 5,000.
Blood culture taken:no b.Typhosus found.
4.7.23. Positive Widal against B. Typhosus. Negative against paratyphoid A and B.
Blood proteins on 28.3.23: Non protein 11% , Albumen 2.9% , Globulin 1.5% . Total protein, 5.4% , of which globulin forms 46% .

## APPENDIX B.

Description of Robertson's Refractometric Method of Determination of the Serum Proteins.

(From Robertson's Manual of Practical Biochemistry.)

# APPENDIX B..

## The Refractometric Analysis of Blood Proteins.

(a) Estimation of the Non proteins.

Glass tubes 25 cm.long,having an inside diameter of 5 mm.and walls about 1 mm.thick,are sealed at one end. It is well to blow gently into the tube while the sealed end is soft,thus making the contour of the bottom of the tube hemispherical and diminishing the tendency to crack on cooling. Into one of these tubes,which has been carefully dried andcleaned,is now introduced exactly 1 cc.of serum with the aid of an accurately calibrated pipette with a capillary tip. Such pipettes may be prepared by taking lengths of narrow-bore glass tubing drawn out to a capillary at one end,introducing 1c.c.of mercury,and marking with a diamond the extremities of the mercury column. The mercury is then delivered into another similar tube,which is similarly marked,and the operation repeated until you have the desired number of pipettes. Prepared in this way your pipettes all deliver (betwween the marks)the same volume of fluid,and if this is the case the exact volume employed is immaterial provided it is in the neighbourhood of 1c.c..

In delivering the serum avoid wetting the upper part of the tube and the formation of air-bubbles.

The serum having been deliveredwith another pipette calibrated against the first deliver 1 c.c.of N/25 acetic acid solution,which may be made up with sufficient accuracy by diluting 4 c.c.of glacial acetic acid to 1750 c.c..

A short length of thick platinum,silver,or nickel wire is now introduced into the tube and the upper end is sealed off in a flame,taking care not to heat the contents. After cooling the tube is shaken and the short length of wire contained in it brings about a thorough mixingof the contents. The tube is now placed in a beaker of cold water of such a depth as to immerse the top of the contained column of liquid several millimetres below the surface. It is well to rest the bottom of the tube upon a wad of glass wool or a piece of wire gauze to avoid the cracking of the tube by bumping during the subsequent boiling. The water is now slowly heated to boiling and allowed to boil energetically for exactly two minutes. The tube is then removed from the boiling water and allowed to cool to room temperature.

When cool the tube is broken open a little above the surface of its contents and the coagulum is broken up. This is best accomplished with the aid of a platinum wire about 0.6-0.7mm. in diameter and provided with several slight bends. This is inserted into the tube and the upper end twirled between the thumb and forefinger. The fluid and the coagulum are now separated by centrifugalization,and the fluid is withdrawn by the aid of a dry,clean pipette,and the refractive indices of the fluid and of N/50 acetic acid solution(prepared by diluting N/25 acetic acid used above to exactly one-half with distilled water)are determined simultaneously. By determining the refractive indices of the fluid and of N/50 acetic acid simultaneously,the necessity for regulation of the temperature at which the readings are made is obviated,for although the absolute values of the refractive indices are affected by temperature the difference between them are independent of the temperature at which they are determined. In carrying out a large number of estimations,however,it is necessary to elimate the possible error due to progressive changes in the temperature of the dark-room by redetermining the refractive index of the solvent (in this instance N/50 acetic acid)at frequent intervals.

The refractive index of a 1 per cent.solution of NaCl is 0.00160 greater than that of distilled water. The refractivities of 1 per cent.solutions of other inorganic salts and of glucose and urea are of very similar magnitude. To within a sufficient degree of accuracy,therefore,the percentage of non-protein substances in the serum may be estimated by dividing their refractivity by the factor 0.00160. Your result must be multiplied by 2,because you have diluted the serum to one-half with N/25 acetic acid.

APPENDIX B..

For the determination of the refractive index, employ a Pulfrich refractometer, which reads the angle of total reflection to within one minute. A sodium flame is used as the source of light. Determine the refractive index from the angle of total reflection with the aid of the table supplied.

When it is desired to make a number of successive determinations, the cup of the refractometer should be carefully dried with absorbent cotton and lens paper before a new sample of fluid is introduced.

(b) Estimation of the Albumins.

Glass tubes 9-10cm. long are prepared, having an inside diameter of 5mm. and one end closed. Tubes which have been employed in the estimation of the non-protein constituents, after having beeen cleaned and dried, may be cut down to the proper length and utilized for this purpose. Small corks or short pieces of glass tubing sealed at one end, the sealed end being pressed against the open end of the longer tubes and held in position by short pieces of rubber tubing, are employed as stoppers. Into one of these tubes is introduced, with the aid of a pipette similar to that described above, 0.5c.c. of saturated solution of ammonium sulphate (prepared by disolving an excess of ammonium sulphate in hot water and allowing the excess to crystallize out on cooling). With another pipette which has been calibrated against the first, introduce 0.5c.c. of serum, drop in a piece of platinum, silver, or nickel wire, affix the stopper and shake thoroughly with as little delay as possible. It is necessary to introduce the ammonium sulphate first, as otherwise, being of greater specific gravity than the serum, it sinks through the serum, portions of which are thus exposed for some time to ammonium sulphate of higher concentration than one-half saturated. This leads to a precipitation of albumins which do not readily redissolve, and the results obtained are erroneous and irregular. If the ammonium sulphate is introduced first the serum floats on top of it and energetic shaking brings about almost immediate admixture of the serum and the reagent. It is well, while shaking, to hold the thumb against the bottom of the tube, thus diminishing the danger of cracking the tube by the impacts of the heavy piece of wire.

The tube, with the stopper still affixed, is now centrifuged. The precipitate soon settles, and sufficient supernatant fluid may be drawn off to fill the tip of a pipette and the space between the two marks known to hold about 0.25c.c. (prepared as described above). This quantity of the supernatant fluid is delivered into another clean, dry tube of the type employed in the precipition, and 0.25c.c. of distilled water is added with the aid of a pipette calibrated against the first. A piece of wire is dropped in, a clean stopper affixed, and the contents are shaken. We now determine the refractive index of this fluid and that of one-quarter saturated ammonium sulphate solution prepared (and kept as a stock solution) by mixing equal volumes of saturated ammonium sulphate solution and distilled water and adding to this mixture an equal volume of distilled water. The difference between these refractive indices represents one-fourth of the combined refractivities of the albumins and of the non-protein constituents. Multiplying by 4, therefore, and subtracting the refractivity of the non-protein constituents we have refractivity of the albumins. Dividing this by 0.00177, we obtain the percentage of albumin in the serum.

(c) Estimation of the Globulins.

Determine the refractive index of the serum and that of distilled water. Subtracting from the difference the known refractivity of the non-proteins and the known refractivity of the albumins, you obtain the refractivity of the globulins. Dividing this by 0.00229, you obtain the percentage of globulin in the serum. Adding together the percentages of albumins and globulins you obtain the percentage of total proteins.