THE PLASMA PROTEIN EQUILIBRIUM FACTOR: A NEW CHEMICAL DETERMINATION, OF CLINICAL SIGNIFICANCE

by

JOHN HARKNESS

*Biochemical Department, Royal Infirmary, Sunderland (England)**

In this communication there will be described a new factor which can be used where the estimation of the plasma proteins is made in the study of the systemic reaction to organic changes in the body. This factor has proved to be more reliable and more informative than the classical albumin/globulin ratio.

The new factor is related to the equilibrium constant of the reaction which depends on the hypothesis that in plasma, in addition to free albumin, globulin and fibrinogen, there is a "combined-protein" molecule of bound albumin-globulin-fibrinogen, the four molecules being assumed to be in equilibrium.

INTRODUCTION

The existence of a combined-protein molecule in plasma has been postulated by many writers and denied by others.

When we failed in our endeavours to obtain a formula by which to correlate a physical property (viscosity) with the plasma chemical composition and especially the protein fractions, we again considered the possibility of the existence of such a combined-protein molecule. During the analysis of the experimental results from this aspect a new factor was derived whose clinical significance was soon apparent.

The details of the analysis have been given in a previous publication (HARKNESS AND WHITTINGTON)[1]: only the essentials are repeated here.

(The plasma proteins were fractionated by the classical sodium sulphate method of HOWE[2], the nitrogen being estimated by a micro-Kjeldahl technique. The fact that these fractions were not pure albumin and globulin was fully realized, but this was the best method available to us. For simplicity, at this stage, the subfractions are ignored and the fractions regarded as homogeneous.)

In support of the hypothesis of the existence of a combined-protein molecule, there is evidence that the fractions behave as would be required by the law of mass action.

If, in serum, as in equation (1), n_1 molecules of free albumin and n_2 molecules of free globulin are in equilibrium with n_3 molecules of bound albumin plus bound globulin,

$$n_1[A_f] + n_2[G_f] \rightleftharpoons n_3[A_b + G_b] \tag{1}$$

then the law of mass action would require that the second equation be satisfied

$$\left[\frac{A_f}{M_A}\right]^{n_1} \times \left[\frac{G_f}{M_G}\right]^{n_2} = k\left[\frac{A_b + G_b}{M_P}\right]^{n_3} \tag{2}$$

* Present address: *Central Laboratory, Portsmouth (England).*

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where k is the true "equilibrium constant" of the reaction and M_A, M_G, and M_P are the molecular weights of the albumin, globulin and combined-molecule respectively.

By the introduction of further assumptions, this can be reduced to equation (3)

$$A^{\alpha} \cdot G^{1-\alpha} = K (A + G) \qquad (3)$$

where A and G are albumin and globulin as measured in g per 100 ml as fractionated.

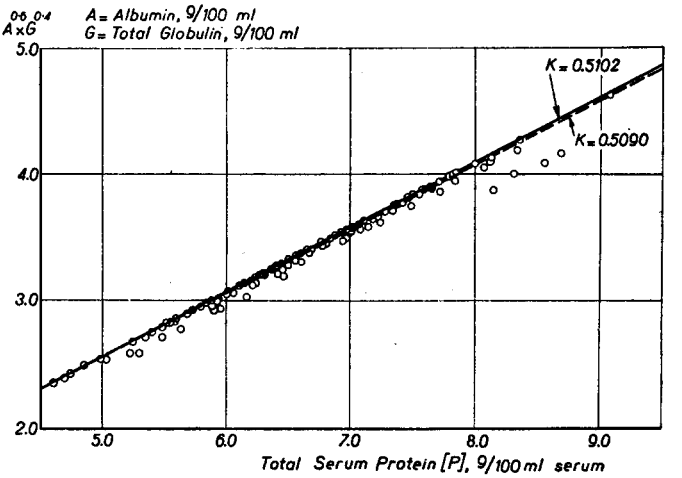

Fig. 1. The serum-protein line (α = 0.6 empirical)

If the hypothesis is correct, then there should be a value for 'α' which would allow K to be approximately constant, and such a value has been found where α = 0.59

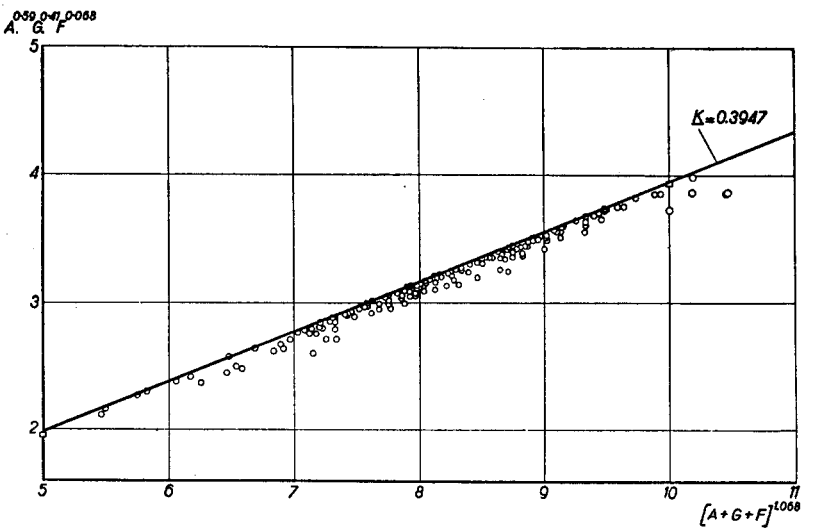

Fig. 2. The plasma-protein line

(empirically α = 0.60, but calculation showed that theoretically α should equal 0.59).

Fig. 1 shows $A^{\alpha} \cdot G^{1-\alpha}$ plotted against (A + G) for 212 tests, and the line K is obviously nearly a straight line, as required by the hypothesis.

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The hypothesis was then extended to plasma, with the equilibrium equation becoming as in (4)

$$A^{\alpha} \cdot G^{1-\alpha} \cdot F^{\beta} = K\,(A + G + F)^{1+\beta} \qquad (4)$$

The diagram for plasma corresponding to Fig. 1 for serum, is shown in Fig. 2, and here it is seen that K is more complicated. While there appears to be an upper limiting value for K at 0.3947, there are numerous and considerable departures from this line.

Consideration of our clinical material soon showed that there was a relation between the clinical condition and the departure of the K value from the straight line.

The clinical material consisted of cases in which the plasma proteins were changing as part of the systemic reaction to organic changes in the body, *e.g.* tuberculosis, sepsis, rheumatism, malaria, malignant growths, etc. Those conditions in which the proteins vary as a result of primary liver or kidney lesions are omitted as insufficient numbers of such cases have been studied to warrant an opinion.

ESTIMATION OF K

When the values of A, G, and F are known (in g/100 ml) K can be calculated from equation (5)

$$K = \frac{R^{\alpha+\beta} \cdot S}{[R + S(1 + R)]^{1+\beta}} \qquad (5)$$

where $R = A/G$, $S = A/F$, $\alpha = 0.59$ and $\beta = 0.068$.

To eliminate this calculation, the diagram of Fig. 3 was constructed whereby K can be read off by interpolation when R and S are known.

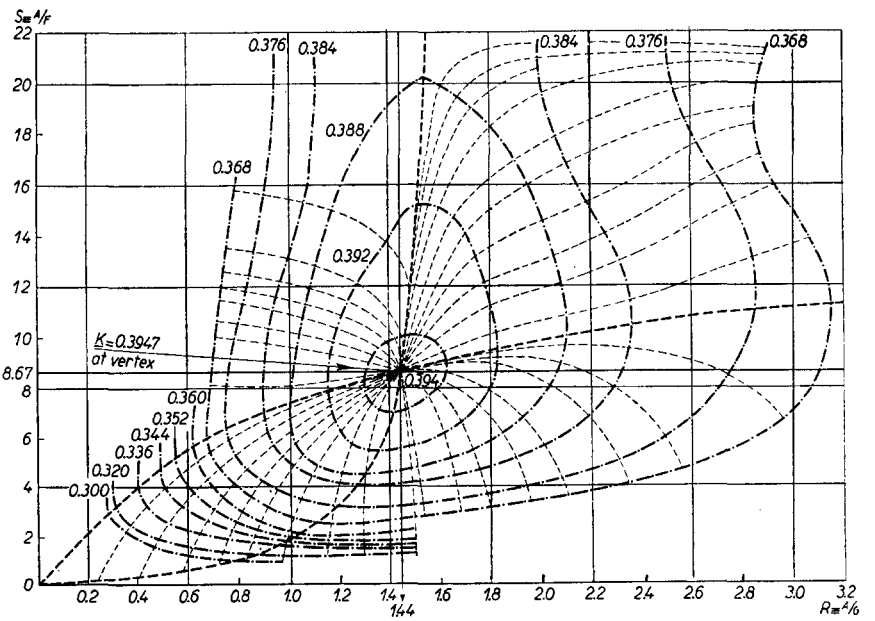

Fig. 3. Nomogram for estimation of K from $R \equiv A/G$ and $S \equiv A/F$. Contours of equal K values Orthogonals of maximum change in K with least change in protein fractions.

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Fig. 3 has also been of great assistance in the understanding of the changes in K and in the differentiation between K values of equal numerical value but different clinical significance.

(Fig. 3 can best be appreciated by regarding it as similar to a contour map, with contours here of equal K values and the peak at 0.3947. Drawn at right-angles to the contours are the orthogonals.)

RELATION OF K TO THE CLINICAL CONDITION

In health, R (= A/G) and S (= A/F) are both high and K values lie in the upper right portion of Fig. 3 and the K values are numerically low.

Generally, it appears that as a response to the onset of infection and organic bodily changes the fibrinogen and/or globulin increases while the albumin later decreases. These changes cause K to move to the left and downwards, and K first *increases* numerically and then *decreases*.

With clinical improvement, K retraces its path towards the right, increasing numerically and then decreasing.

From our experience in about 285 cases, we believe that the introduction of the A/F ratio has produced in the K factor a value which is superior to the A/G ratio as an indication of the reaction of the body to associated organic changes.

a. Outside the upper right section of normality, cases of equal clinical severity were associated with approximately the same numerical value of K although the situation (and A/G ratio) in Fig. 3 could be highly variable.

This point is well exemplified by Fig. 4 in which the average K values are plotted

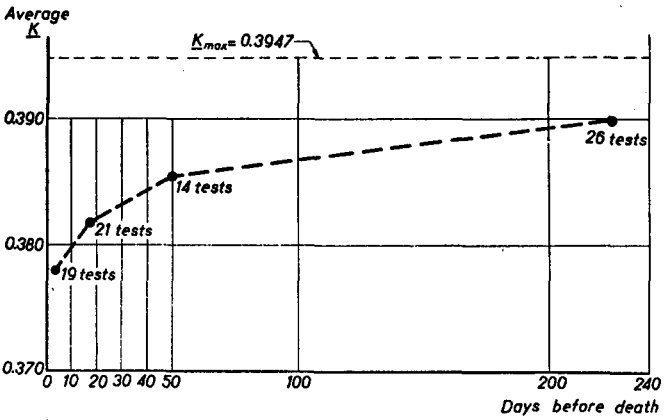

Fig. 4. Terminal fall in K, the plasma-protein equilibrium factor

against the average interval prior to death. K thus falls steadily with the approach of death (and increased clinical severity). In several instances the A/G ratio even increased prior to death (see case 1).

Also, with the exception of two cases of rheumatoid arthritis who were sufficiently ill as to require admission to hospital, no case recovered where K fell below 0.380 in the left upper and lower sections of Fig. 3. Admittedly the A/G ratio in most of these cases was also low, but in several it was as high as 1.5 which is not regarded as of fatal significance.

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b. The orthogonals, drawn at right-angles to the contours, represent the paths along which a plasma must move in order to achieve the maximum change in K with the least change in the actual values of the protein fractions — just as the quickest way of descending a hill is to move at right-angles to the contours.

These orthogonals are not just fanciful insertions and attain significance when it can be shown that serial results tend to move along them during progress and recovery in some cases (see case 3).

In many instances the clinical changes could be represented equally well by the changes in the simple A/G ratio but attention is drawn to that part of Fig. 3 where A/G equals about 1.2 to 1.5, where the orthogonals are almost vertical. Here are found cases (such as case 3) in which decrease in K truly reflects the worsening of the clinical condition while the A/G ratio remains almost unchanged.

It will be realized that the orthogonals actually inserted into Fig. 3 are only a few of the possible infinite number of such lines.

c. All cases do not follow the orthogonals however, *i.e.*, the rate of change is not maximal. In these, the serial results may fall on Fig. 3 so that the K values follow regular definite trends: or the serial results may appear to fall at random on Fig. 3 yet more careful analysis shows that the K values have a regular increase or decrease numerically which correspond to the changes in the clinical condition. While the A/G ratio may increase and decrease irregularly from day to day, the introduction of the A/F ratio is such as to correct for these irregularities and produce a regular even alteration in the K values.

This again illustrates the superior reliability of K compared with the A/G ratio.

d. In some instances (as in case 2) it may be found that the ultimate clinical outcome can be indicated by the factor K before any other empirical test, such as the E.S.R. or the plasma viscosity. In case 2, K retreated from the threshold of fatal values (0.380) while the plasma viscosity was still rising.

e. The K factor is being used in search of malignant lesions in parts of the body not readily available to direct investigation. Where such a condition is suspected, K (and more especially serial K evaluation) frequently confirms its presence by indicating a reaction to organic changes in the body while other tests (E.S.R., plasma viscosity, etc.) are negative or ambiguous (see case 4). Wide infiltration by a malignant tumour may be associated with insignificant changes in K in a few cases, yet, if it is remembered that the test is non-specific and if other causes of systemic reaction can be excluded, the proportion of cases in which the estimation of K is of real assistance to the clinician makes the test worth performing.

In serial estimations, a movement of K to the left and downwards would favour a diagnosis of malignancy; and a K moving to the right and upwards would be strongly against such a diagnosis (see case 5).

CASE HISTORIES

Five case histories only are presented. They include most of the points made out in the general statements above. The results are summarized in Table I.

1. Case A.B.; senile patient with gangrene of feet and extensive bed sores.

This case illustrates the steady decrease in the K values as death approaches, without a corresponding consistent change in the A/G ratio.

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2. Case I.R.; elderly patient with phthisis.

The *K* value here shows the ultimate outcome correctly and earlier than the other two tests. The *K* value increases, indicating a reduction in the systemic reaction to the tuberculous focus, while the plasma viscosity is still increasing. In this instance the A/G ratio would have been equally informative and the E.S.R. parallels the change in *K*.

3. Case B.R.; acute rheumatic fever in young man.

This case is especially instructive.

Following a sore throat, the patient developed a classical attack of rheumatic fever, for which he was admitted to hospital. Sodium salicylate was pushed for three weeks, with only a partial response, when the drug had to be discontinued on account of toxic symptoms. Calcium aspirin was given as a substitute.

Test No. 1 was performed on the day before the salicylate was stopped. The stoppage was followed by an immediate relapse in the clinical condition as indicated by elevation of temperature, increased sweating, increased joint pains, etc. This deterioration is reflected in the movement of *K* down an orthogonal, while there is no significant change in the A/G ratio: the plasma viscosity increase parallels the change in *K*, but the E.S.R. shows a definite decrease.

Three days after test No. 2 sodium salicylate treatment was resumed; two days later still, some septic teeth were removed while the patient was receiving large prophylactic doses of penicillin. Clinical improvement thereafter was steady so that he was discharged two weeks later to continue resting at home.

Tests Nos. 3, 4, and 5 show the movement of *K* approximately along an orthogonal while the A/G ratio shows no change. The changes in the plasma viscosity again parallel those of *K*, but the E.S.R. changes are more erratic.

Test No. 6 was performed at his first attendance as an out-patient. There was no evidence of cardiac involvement.

4. Case J.H.; non-detectable malignant growth.

This patient, a man of 57 years, consulted his doctor in October 1946 with a history of some months loss of weight, cough, rectal bleeding and vague abdominal pains. After a thorough investigation in hospital, including barium meal, pyelograms, etc., no organic lesion could be detected apart from bleeding piles which were dealt with surgically. There was an evening elevation of temperature. Malignancy was considered, but no primary growth could be detected. A diagnosis of Periarteritis Nodosa was also considered possible although this was not confirmed by muscle section.

He was readmitted in January 1947 on account of increasing anaemia. *K* was estimated at this time and its very low value indicated a severe systemic reaction to an organic change, with a fatal outcome highly probable. Thorough investigation still failed to reveal this organic lesion.

Later he was investigated at another centre where a diagnosis of Aleukaemic Leukaemia was made. However, two weeks before his death in May 1947, he suddenly developed haematuria and a swelling in the lumbar region which left no doubt as to the true diagnosis of 'Hypernephroma'.

5. Case K.S.; pathological fracture in middle-aged patient.

This case illustrates the value of serial estimation of *K*.

The patient was in rather poor general health; there was vomiting frequently after meals. While moving in bed, the right femur fractured. Skiagrams showed another small area of rarifaction in this femur, sclerosis of the other femur, and a mottling of the skull

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TABLE I

Case	Test No.	Date of death	Date of test	A	G g/100 ml	F	A/F	A/G	K	Corrected Citrate Plasma Viscosity	Maximum Citrate E.S.R. (mm/h)
1	1	24/3/44	13/3/44	3.64	2.14	0.91	3.99	1.70	0.380	1.837	225.0
A.B.	2		15/3/44	3.55	2.54	1.02	3.47	1.40	0.376	1.961	540.0
	3		21/3/44	3.64	2.48	1.11	3.25	1.46	0.371	2.041	420.0
2	1	—	4/12/43	2.75	3.15	0.54	5.06	0.87	0.380	1.663	90.0
I.R.	2		31/1/44	3.17	3.35	0.38	8.31	0.95	0.386	1.720	46.8
			22/2/44	3.33	3.29	0.49	6.75	1.01	0.3882	1.738	33.2
			11/8/44	3.99	3.05	0.40	9.73	1.31	0.3936	1.715	33.3
3	1	—	12/8/47	3.62	2.90	0.56	6.43	1.25	0.393	1.639	55.7
B.R.	2		18/8/47	3.39	2.80	0.72	4.68	1.21	0.3887	1.682	41.2
	3		25/8/47	3.65	2.37	0.66	5.54	1.54	0.3905	1.647	43.5
	4		4/9/47	3.94	2.54	0.55	7.20	1.55	0.3938	1.614	35.1
	5		11/9/47	3.81	2.48	0.47	8.14	1.54	0.3943	1.575	28.8
	6		9/1/48	4.72	1.96	0.37	12.82	2.41	0.382	1.530	7.4
4	1	May '47	4/2/47	3.80	3.69	1.51	2.52	1.03	0.359	—	—
J.H.											
5	1	—	1/4/47	3.99	2.66	0.60	6.65	1.50	0.393	1.695	48.4
K.S.	2		15/4/47	4.43	2.70	0.56	7.92	1.64	0.3942	1.664	96.0

Case 1 (A.B.) = senile with gangrene of feet and extensive bed sores
,, 2 (I.R.) = elderly patient with phthisis
,, 3 (B.R.) = acute rheumatic fever in young adult
,, 4 (J.H.) = malignant growth, non-detectable clinically
,, 5 (K.S.) = pathological fracture of femur, ? cause

which was reported as consistent with secondary malignant deposits. In addition to those shown in Table I, the following laboratory results were obtained:

TABLE II

Serum Calcium	= 16.3 mg/100 ml
Serum Inorganic Phosphate	= 3.9 mg/100 ml
Serum Acid Phosphatase	= 2.3 K.A. units/100 ml
Serum Alkaline Phosphatase	= 19.5 K.A. units/100 ml

The consensus of several opinions was that this was a case of gastric carcinoma with secondary bone deposits. Treatment was symptomatic, chiefly directed at relieving the vomiting.

The picture was changed entirely when K was again estimated two weeks later. K now indicated an improvement. An improved K, especially when produced mainly by an increase in the albumin fraction, was inconsistent with a diagnosis of malignancy. A revised diagnosis of a simple parathyroid adenoma was confirmed at operation.

(Support of the diagnosis of non-malignancy was given by the perfect relationship of the E.S.R. and the plasma viscosity; also, the phenomenon in the plasma viscosity which has been called "Terminal decay" by HOUSTON, HARKNESS, AND WHITTINGTON[3] was absent.)

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DISCUSSION

Since its introduction by VAN SLYKE, the A/G ratio has been studied in a wide variety of clinical conditions and considerable information has been gained. More recently the tendency has been to study the absolute values of the fractions and less reliance has been placed on the A/G ratio. The weakness of the A/G ratio lies in the fact that it presents only a part of the protein change which is going on in the blood and apparently the fibrinogen cannot be neglected if a true stereoscopic picture is to be obtained.

In support of the contention of the importance of the fibrinogen, it may be noted that the present writer found a poor correlation between the physical properties of serum and the clinical condition, in contrast to the excellent correlation between the clinical state and the physical properties of the plasma (HOUSTON *et al*[3]; HARKNESS *et al.*[4]; COWAN AND HARKNESS[5].)

The concept of the factor K depends on two suppositions, (a) that in the plasma there exists a combined-protein molecule which is in dynamic equilibrium with the free molecules, and (b) the changes in the plasma proteins parallel the systemic reaction to the original change.

The question of the existence of a combined-protein molecule has been and is being discussed by many workers in this field. This combined-molecule may break up very readily so that no method yet devised may estimate it although PEDERSEN[6] has found a combined-protein molecule, his 'X-protein', which has stood up to the strain of the ultracentrifuge although its recovery varies greatly according to the experimental conditions.

It has long been held that an organic change in the body causes a systemic reaction which includes a change in the plasma proteins, and that the degree of change in the proteins parallels the degree of systemic reaction. This is the basis of the E.S.R. test and the various flocculation reactions.

One of these empirical tests, the plasma viscosity estimation, depends entirely on changes in the proteins, the effect of gross changes in the non-protein constituents being negligible. In previous publications a close correlation has been shown to exist between the plasma viscosity (and hence the plasma proteins) and the systemic reaction and the severity of the organic changes.

It would appear to be logical to study the fundamental changes in the plasma protein fractions themselves rather than functions dependent upon these changes. There have been two obstacles to this however.

Firstly, up to the present scheme, there has been no simple method or single numerical value to indicate the changes which are occurring in all three fractions of the plasma proteins. The attempt at producing the results as a single figure in the A/G ratio was defective as it noted only part of these changes and as a result, showed poor correlation with the clinical condition.

Secondly the accurate estimation of the protein fractions is relatively more expensive in time, material and laboratory working space. When compared with the simple empirical tests, the Kjeldahl estimation is a formidable procedure, while the simpler colorimetric, turbidometric, and similar techniques do not yield sufficiently reliable results.

Both of these objections are surmounted by the present procedure wherein the

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K factor indicates the changes in all three fractions and our micro-Kjeldahl technique reduces the Kjeldahl distillation procedures to a total of about 40 minutes.

The factor K is related to the true 'equilibrium constant' of a hypothetical equation of equilibrium between the protein fractions. It is interesting to speculate upon the changes in K as related to SCHOENHEIMER'S[7] ideas of constant change in the molecules and tissues of the body. There is an attraction in the idea of K first increasing as the rate of tissue change is increased to react upon the organic bodily change and then gradually falling as the primary impetus is gone. When recovery begins, K again increases, to fall finally into the lower normal levels. When the processes of the body are so overwhelmed that recovery will not be possible, the condition is reflected in the depression of K below a limiting value (0.380). The factor K and its movement in Fig. 3 assists the clinician in understanding the way in which the patient is reacting to offensive stimuli.

No attempt can yet be made to explain the mechanism which produces these changes in the plasma proteins or the organ or organs which are responsible for the changes. If it is believed that the liver forms the plasma proteins, K would appear to measure a liver function.

CONCLUSIONS

From the hypothesis that in plasma there exists a combined-protein molecule consisting of bound albumin-globulin-fibrinogen in equilibrium with the free portions of the albumin, globulin, and fibrinogen fractions, a factor K is derived which is related to the true 'equilibrium constant'.

A chart (Fig. 3) is presented for the calculation of K from the A/G and A/F ratios.

The factor K varies in disease and the variation is of clinical significance.

K is superior to the A/G ratio as an index of the clinical condition, especially for serial investigation.

K may be a more sensitive index than either the E.S.R. or the plasma viscosity.

Acknowledgements

Credit for the analysis which lead to the derivation of the factor K must go to Mr R. B. WHITTINGTON, M.Sc., of Manchester University.

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I am indebted to the Editor of *Analytica Chimica Acta* for permission to reproduce Figs 1 to 4.

SUMMARY

The Plasma Protein Equilibrium Factor is defined.

A chart is produced by which the factor can be evaluated when the albumin, globulin and fibrinogen fractions of the plasma protein are known.

The relation of this factor to the clinical condition is described and discussed and some representative cases are presented in detail.

It is shown that the estimation of the factor is useful and informative to the clinician; it is a more reliable test than the classical Albumin/Globulin (A/G) ratio.

RÉSUMÉ

Le facteur d'équilibre des protéines du plasma est défini.

Un diagramme est établi, grâce auquel il est possible de calculer ce facteur, lorsqu'on connaît les proportions d'Albumine, de Globuline et de Fibrinogène dans les protéines du plasma.

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Le facteur dépend des conditions pathologiques: quelques exemples de cette dépendance sont étudiés en détail. La détermination de ce facteur est utile au clinicien: elle donne des renseignements plus sûrs que le rapport classique Albumine/Globuline (A/G).

ZUSAMMENFASSUNG

Der Plasmaeiweiss-Gleichgewichtsfaktor wird definiert. Es wird eine Tabelle aufgestellt, aus der der Faktor bestimmt werden kann, wenn die Albumin-, Globulin- und Fibrinogenfraktionen des Plasmaeiweiss bekannt sind.

Die Beziehung dieses Faktors zum klinischen Zustand wird beschrieben und diskutiert; einige repräsentative Fälle werden eingehend beschrieben.

Es wird gezeigt, dass die Bestimmung dieses Faktors für den Kliniker nützlich und aufschlussreich ist; sie ist eine zuverlässigere Probe als das klassische Verhältnis Albumin/Globulin (A/G).

REFERENCES

1 J. HARKNESS AND R. B. WHITTINGTON, *Anal. Chim. Acta*, 1 (1947) 153.
2 P. E. HOWE, *J. Biol. Chem.*, 49 (1921) 93.
3 J. HOUSTON, J. HARKNESS, AND R. B. WHITTINGTON, *Acta Tuberc. Scand.*, 19 (1945) 153.
4 J. HARKNESS, J. HOUSTON, AND R. B. WHITTINGTON, *Brit. Med. J.*, 1 (1946) 268.
5 I. C. COWAN AND J. HARKNESS, *Brit. Med. J.*, 2 (1947) 686.
6 K. O. PEDERSEN, *Ultracentrifugal studies on serum and serum proteins*, Uppsala 1945.
7 R. SCHOENHEIMER, *The dynamic state of body constituents*, Harvard University Press 1942.

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