XXII. A COMPARATIVE INVESTIGATION OF THE CORRESPONDING PROTEINS OF COW AND OX SERUM, COW'S COLOSTRUM AND COW'S MILK BY THE METHOD OF PROTEIN RACEMISATION.

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Method of protein racemisation.

The method has its basis in the initial observations of Kossel [1911, 1912, 1913] and of Dakin [1912] that when solutions of protein in dilute sodium hydroxide solution are kept at 37°, they suffer a progressive diminution in the value of their optical rotatory power. Dakin [1912] attributed this change to a keto-enol tautomerism of the =CH—CO— groups in the protein complex and demonstrated further that the optical characters of the individual amino-acids obtained by hydrolysis of a racemised protein afforded some information as to their situation within the original protein molecule.

Dudley and Woodman [1915] were able to detect structural differences in the amino-acid make-up of the caseinogens of the cow and the sheep by racemising the proteins under comparable conditions, hydrolysing the racemised proteins and comparing the optical properties of the corresponding amino-acids. They were also able to confirm the identity of the euglobulin and pseudoglobulin of cow's colostrum [1918]. Dakin and Dale [1919] demonstrated in a similar manner the existence of structural differences between the albumins of the eggs of the duck and the hen, a result which was confirmed by subsequent biological trials.

A simplified racemisation method.

The main objections which may be urged against the method of protein racemisation referred to above are twofold.

Firstly, like most other methods in protein research, the method involves certain tedious and laborious processes such as hydrolysis and the subsequent separation of the amino-acids by the Fischer-Levene esterification process and the Kossel-Kutscher method for the diamino-acids. Large amounts of material, often only prepared with difficulty, are requisite.

Secondly, the nature of the processes involved prevents the method from being quantitative, and differentiation between proteins is only possible when very sharp distinctions in the optical characters of corresponding amino-acids occur. In addition to this, certain of the amino-acids like proline may undergo partial racemisation during their isolation from the hydrolytic products of the racemised protein, and thus the significance of the optical findings in such cases may be obscured.

In the investigation to be described in the present communication, the racemisation reaction has been utilised for the same purpose in a much simpler manner by following quantitatively, and under perfectly definite conditions, the actual course of the racemisation of certain proteins and thus obtaining what might be termed their "racemisation curves." Preliminary trials carried out by Dudley and Woodman [1918] in their work on the globulins of colostrum had given rise to the hope that it might be possible to establish identity or non-identity of related proteins by such means.

If, for example, the fall in rotation of a 2 % solution of globulin in $N/2$ soda be followed at 37°, it is found that if the specific rotations be plotted against the time in hours during which the reaction has been allowed to proceed, then the readings fall on a perfectly smooth curve of the type shown in Diagram I. The specific rotation of the solution, which initially is about $-80°$, sinks very rapidly at first, then more slowly and subsequently after about 250 hours attains a practically constant value of about $-22°$. If $N/4$ alkali be used instead of $N/2$, the gradient of the curve is less steep. This behaviour is connected with the role played by the alkali in the reaction and this aspect of the question will be discussed in a further communication dealing with the mechanism of the reaction between proteins and dilute alkali at low temperatures. At present it need only be pointed out that the actual significance of the reaction does not materially affect the value of the results obtained by its use in comparing different proteins, since all the trials are carried out under strictly comparable conditions.

On the assumption that each individual protein will possess its own specific set of curves, the racemisation reaction can be made the basis of a method for testing identity or non-identity of proteins. If two proteins are to be pronounced structurally identical, then if racemised under the same conditions, their solutions must show the same initial rotation, the same final rotation and the same rate of diminution of optical rotation; the last named is probably intimately connected with the intramolecular structure of the protein. Not only this, the proteins must continue to display identical optical behaviour when the concentrations of the alkali and of the protein are varied. Thus the test can be made very exhaustive. If the two proteins are not structurally identical, this will be revealed by their possessing distinct sets of racemisation curves.

It must be pointed out, however, that the amount of difference between the optical values of two proteins in dilute alkali is not an absolute measure

of the amount of structural difference existing between them, since the optical rotatory power of a complex substance is by no means a simple additive function of the activities of its several constituents. All that the method can claim to do is to establish structural identity or non-identity, and in this respect it is perhaps superior to the van Slyke method, since it is manifestly possible for two proteins to be quantitatively identical with regard to their amino-acid content, and yet be distinct proteins by virtue of differences in the order of linkage of the amino-acids. The caseinogens of the cow and the sheep afford such an example; these proteins cannot be differentiated by the van Slyke method, but were shown to be different stereochemically by the ordinary protein racemisation method [Dudley and Woodman, 1915]. The van Slyke method and the simplified racemisation method might be most usefully employed in conjunction with each other.

The simplified racemisation method can claim the following advantages, that it is relatively simple and rapid and only requires the use of small amounts of material. It has been employed with complete success in investigating the relationships existing amongst the corresponding proteins of serum, colostrum and milk.

The corresponding proteins of cow and ox serum, cow's colostrum and cow's milk.

An examination of the available data regarding the structural relationships which exist among these proteins serves to reveal the existence of discord between the chemical and biological evidence. The investigation of the structure of a protein by chemical means is manifestly a matter of extreme difficulty. It is possible to determine quite readily, and with a fair degree of accuracy, the *amounts* of the various amino-acids in any given protein by the van Slyke distribution method; but the study of the precise *order* in which those amino-acids are linked up within the protein molecule presents a baffling problem, even in the case of simple peptone derivatives.

It is scarcely a matter of surprise, then, that in the past, the chemist has had to rely largely on the biologist to furnish him with information regarding the relationships existing among any given class of proteins. The biologist has been able to pursue this line of enquiry with the help of certain well-known methods, such as the precipitin and anaphylaxis methods, which have been evolved by workers on immunity.

In this way he has been able to draw the following conclusions. Firstly, that the globulin of serum is identical with the globulin of colostrum. Secondly, that the albumins of serum, milk and colostrum are one and the same protein. Hamburger [1901], by the use of the precipitin method, demonstrated the existence of a very close relationship between the proteins of blood and milk. Further confirmation of this was derived from the work of Schlossmann and Moro [1903], who concluded that the albumin of the blood was identical with lactalbumin and because of this were inclined to the assumption that the

infant, in the early days of its existence, may be able to absorb directly the milk albumin. Bauer and Engel [1911], after testing the question by means of the method of complement deviation, also came to the conclusion that the serum proteins were identical with the corresponding proteins of milk and colostrum.

The identity of the proteins of serum and milk, as established by biological methods, has led physiologists to make the following generalisation: That it is not necessary to assume a distinct mammary synthesis for the elaboration of the albumin and globulin of the mammary secretion, but that these are present in the colostrum and milk as a result of a simple and direct transference from the blood stream.

The results of chemical investigation, however, are not wholly in harmony with the above findings. Several earlier workers, as a result of work based on simple chemical analysis and a study of the methods of precipitation, drew conclusions in accord with those obtained in the biological investigations. Such evidence, however, is of little use in drawing conclusions regarding the structure of the protein molecule.

With regard to the globulins, the chemical work amply confirms the results obtained by biologists in demonstrating the identity of lactoglobulin and serum globulin. Conclusive evidence was furnished by the work of Hartley [1914] on serum globulin and of Crowther and Raistrick [1916] on the globulin which can be isolated in large amounts from colostrum and in very small amounts from normal milk. These investigators analysed the proteins by means of the van Slyke method, and a comparison of their data fails to reveal any differences in the quantitative nature of the amino-acid make-up of the various globulins.

In respect of the albumins, however, chemical evidence is available which would suggest that lactalbumin and serum albumin, far from being identical, are two distinct proteins. As far back as 1885, Sebelien [1885] prepared lactalbumin and serum albumin by similar methods and found that whereas lactalbumin from milk or colostrum has a specific rotation in water of about $-37°$, that of serum albumin is about $-63°$. Later, Halliburton [1890] showed that lactalbumin differed from serum albumin in its behaviour on heat coagulation and with regard to its precipitability by neutral salts. Referring again to the work of Hartley and of Crowther and Raistrick, a comparison of their van Slyke data for the two albumins reveals marked differences in respect of their quantitative amino-acid content. Differences occurred in every item of the analyses, but were especially marked with regard to the basic nitrogenous constituents.

The present work, therefore, was undertaken partly with a view to investigating this discrepancy between the biological and chemical findings and partly with the object of testing the applicability of the simplified racemisation method to problems of this type.

It will be convenient at this stage to summarise the main results of the

investigation. Firstly, the euglobulin and pseudoglobulin fractions in both serum and colostrum have been shown to be identical proteins. Secondly, serum globulin has been found to be identical with colostrum globulin. Thirdly, as one would anticipate, no differences were detected between the corresponding proteins of cow and ox serum. Fourthly, the albumins of milk and colostrum were found to be the same individual protein. Fifthly, lactalbumin and serum albumin have been shown to be distinct chemical individuals and it is reasonable to speculate whether, by the use of the more modern and refined anaphylactic methods, it would not be possible to obtain evidence in harmony with the chemical results as to the non-identity of the two albumins.

If the chemical results are to be accepted, it must be assumed that a distinct mammary synthesis is necessary for lactalbumin as well as for caseinogen; and it would appear that the temporary power of the organism of permitting relatively large amounts of serum globulin to be transferred from the blood stream to the colostral secretion is of the nature of an emergency measure. From the results of Crowther and Raistrick [1916] it would further appear that even when normal lactation sets in, the serum globulin can leak, as it were, in extremely small amounts into the milk secretion. The basal nitrogenous constituents of normal milk, namely caseinogen and lactalbumin, are elaborated by the mammary gland both in the colostral and the normal lactation periods.

Preparation of the proteins.

For the purposes of comparative work, it was essential to prepare the proteins from the different sources by the use of the same methods, since it was at least possible that the chemical composition of a protein preparation might vary within limits, if the method of isolation were varied. Experiments will be described later, however, in which it was found impossible to detect, by the use of the simplified racemisation method, differences between two samples of globulin, which had been prepared by two absolutely different methods.

The total globulin was removed from neutral solution by half-saturation with ammonium sulphate; the pseudoglobulin and euglobulin were separated by dialysis. The albumin was obtained from the filtrate from the total globulin by the addition of the requisite volume of $N/5$ sulphuric acid together with an equal volume of saturated ammonium sulphate solution, in order to maintain half-saturation. The albumin was freed from ammonium sulphate by dialysis.

The samples of protein relied on had all been fractionally precipitated seven times, and in order to be sure that further precipitation would not affect the optical behaviour of the protein in dilute alkali, portions of protein from successive fractionations (namely, the sixth and the seventh) were racemised separately. In this way, it was possible to obtain a check on the completeness of the separation of the albumin from the globulin.

Proteins of ox serum.

Globulin. Fresh ox blood was defibrinated and centrifuged without delay. After filtering through cotton wool, the serum was diluted with its own volume of water and an equal volume of saturated ammonium sulphate solution was added. After standing several hours, the precipitate of globulin was filtered off on a large Buchner funnel and the filtrate was used for the isolation of the albumin. The crude globulin was redissolved in distilled water and the bulk made up to that of the original diluted serum. After filtering the solution, an equal volume of saturated ammonium sulphate solution was again added and the precipitated globulin was filtered off. The precipitation of the globulin was carried out in all seven times; a small portion of the precipitate after the sixth fractionation, however, was retained for comparison with the globulin obtained at the seventh precipitation. The final solution of globulin was filtered and dialysed in large conical shaped parchment dialysers (which had previously been immersed in a 5 % solution of gelatin and then submitted to the action of formalin vapour). After dialysing for several days in running tap water, the process was continued in frequent relays of distilled water, until the solution was free from sulphate. In this manner, the globulin was separated into pseudoglobulin, which remained in solution, and euglobulin, which separated out during dialysis.

The euglobulin was filtered off from the pseudoglobulin and was dissolved in the minimum amount of 0·6 % NaCl solution. After filtering the solution, the euglobulin was reprecipitated by the addition of about fifteen times its volume of distilled water. The precipitate settled on standing and the bulk of the water was decanted off. After filtering on to a Buchner funnel, the euglobulin was washed with distilled water and finally dried with alcohol and anhydrous ether.

The filtered pseudoglobulin solution was cooled in ice and the protein precipitated by the addition of ice cold alcohol. The precipitate was filtered off and dried with alcohol and anhydrous ether. The amount of pseudoglobulin obtained in this process was appreciably greater than that of the euglobulin.

Two per cent. solutions of pseudoglobulin obtained as a result of six and seven fractionations respectively were racemised by means of $N/4$ NaOH. The following readings are taken from the series of polarimetric determinations made on the solutions from time to time during the course of the racemisation.

Pseudoglobulin after 6 precipitations 2 % in $N/4$ NaOH		Pseudoglobulin after 7 precipitations 2 % in $N/4$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
1½	−75·5°	1½	−76·0°
26	−54·5	24	−55·0
48	−48·5	53	−48·0
96	−42·0	93	−42·0
144	−38·0	140	−38·0

Similar agreement was obtained when 2 % solutions of these samples in $N/2$ alkali were racemised and from this it was concluded that after six precipitations a definite and individual globulin preparation had been isolated, whose composition and properties were unaffected by further fractionation.

Albumin. To the filtrate from the total globulin was added the requisite volume of $N/5$ sulphuric acid to effect precipitation of the albumin, the necessary amount for complete precipitation being previously determined on a small bulk of solution. After allowing to stand for 24 hours, the albumin was filtered on to a large Buchner funnel. It was then redissolved in distilled water and made exactly neutral by means of very dilute caustic soda; the solution was filtered, made up to the original volume and to it was added an equal volume of saturated ammonium sulphate solution. The small amount of globulin which separated was filtered off and added to the bulk of crude globulin. The albumin was precipitated from the filtrate by once more acidifying with the requisite amount of $N/5$ sulphuric acid. This process of fractionation was carried out in all seven times. After the fifth fractionation, no globulin precipitate was obtained when ammonium sulphate was added to the neutral solution.

The final albumin precipitate was dissolved in water after being well washed with saturated ammonium sulphate solution, and the filtered solution was submitted to exhaustive dialysis until free from sulphate. The dialysed solution was filtered and cooled in ice, and the albumin was precipitated with ice cold alcohol. It was subsequently dried by means of alcohol and anhydrous ether and was obtained as a white powder, soluble in water.

It was found, as with the globulin, that samples of albumin from the sixth and seventh fractionations displayed identical optical behaviour in $N/2$ and $N/4$ caustic soda.

Proteins of cow serum.

The globulins and albumin were isolated from fresh cow's blood by exactly the same methods used for the preparation of the corresponding proteins from ox blood. The preparations were the result of seven fractional precipitations. As with ox serum, the amount of pseudoglobulin obtained from the cow serum was very much greater than that of euglobulin.

Proteins of colostrum.

For the purposes of this investigation, the first drawn milk from two cows after parturition was collected and mixed. The colostrum was diluted with twice its bulk of distilled water and the caseinogen was precipitated by the addition, drop by drop, of 50 % acetic acid until complete precipitation had occurred. During the acidification, the bulk of liquid was vigorously stirred in a tall glass jar. The caseinogen was allowed to settle and the clear supernatant liquid was siphoned off; the residual precipitate of caseinogen was filtered off and the filtrate was added to the bulk of the solution of globulin

and albumin. The latter was then filtered, made exactly neutral with dilute caustic soda and the proteins were isolated by precisely the same methods as have been described for the separation of the serum proteins. They were obtained as white powders and had all been fractionally precipitated seven times. The colostrum pseudoglobulin was found to preponderate considerably in amount over the euglobulin.

The albumin of milk.

The isolation of globulin from milk was not attempted. Mixed milk from several cows was diluted with twice its bulk of distilled water and the caseinogen was separated in the manner already described for colostrum. The subsequent isolation of the lactalbumin was carried out by the method used for the serum and colostrum albumins. It was purified by seven fractionations, and after drying with alcohol and anhydrous ether, gave a white voluminous powder completely soluble in water. Tests carried out on small portions of albumin from the sixth and seventh fractionations showed no difference in optical behaviour with either $N/2$ or $N/4$ NaOH.

DETAILS OF METHOD FOR FOLLOWING COURSE OF RACEMISATION OF PROTEINS IN DILUTE ALKALI AT 37°.

Before investigating the behaviour of the proteins in dilute alkali, the samples were finely ground up and then dried *in vacuo* over calcium chloride for several days. The determinations were carried out in the following manner. Exactly 1 g. of dried protein was weighed out into a 50 cc. flask containing a little distilled water. The flask was then shaken gently to bring the protein into solution or suspension. 25 cc. of N NaOH free from carbonate (or $N/2$ as the case may be) were then run in from a pipette, and after mixing gently, the volume was made up almost to the 50 cc. mark with distilled water. The flask was then placed in the incubator at 37° and after the contents had become warm, the volume was made up exactly to the 50 cc. mark. After a short time, the solution was filtered quickly into a small dry flask, which was then stoppered and kept in the incubator. From time to time, determinations of the optical rotation were made in a 1 d. polarimetric tube, using a Schmidt and Haensch instrument and sodium light. This procedure was continued for about 300 hours, when the value had become practically constant.

Graphs were then constructed showing the progress of racemisation. The ordinates represented specific rotation values and the abscissae the number of hours during which the reaction had been allowed to proceed.

It was found inadvisable to attempt to dissolve the proteins directly in the dilute alkali, as the substances show a tendency to become coated with glutinous material, which retards the dissolving process. The experience of this investigation showed that in work of this type it would be more convenient not to precipitate the proteins from solution by means of alcohol,

but to deal with solutions of protein which had been concentrated considerably *in vacuo* at low temperature. With preparations of this kind, not the slightest difficulty is experienced in readily making up solutions of known concentration in alkali.

Such perfectly clear and only very slightly yellow solutions of protein in the dilute alkali ($N/2$ and $N/4$ NaOH) have been preserved over periods of months without the slightest indications of bacterial decomposition.

The investigation of each sample of protein was carried out at least in duplicate; the duplicate sets of readings showed very satisfactory agreement. In most cases, however, three and even four series of optical readings were taken in order to determine the positions of the various racemisation curves with the fullest possible degree of certainty. The experimental error in reading with a Schmidt-Haensch instrument is very small, and with practice the reading in a 1 d. tube could be determined to 0·01°. Thus the specific rotation of a 2 % solution of protein could be relied on to 0·5°.

Racemisation of the globulins.

The figures obtained in the racemisation of the various globulins are given in Table I and Diagram I. The data in each case represent the progress of the racemisation up to about 250 hours, when the value of the specific rotation had attained a practically constant value. After this, however, the rotation still underwent a very small, but quite definite diminution in value. Discussion of this point, however, will be left to a future communication.

Table I. *Figures obtained in the racemisation of the various globulins.* (*The observations for the pseudoglobulins of cow serum and colostrum are given in Diagram I.*)

1		2		3		4	
Ox serum pseudoglobulin. 2 % in $N/2$ NaOH		Cow serum euglobulin. 2% in $N/2$ NaOH		Ox serum euglobulin. 2 % in $N/2$ NaOH		Cow colostrum euglobulin. 2 % in $N/2$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation	Time in hours	Specific rotation	Time in hours	Specific rotation
1½	−74·5°	1½	−74·0°	2	−72·5°	1	−75·5°
22	−48·0	24	−47·5	7	−62·0	25	−46·5
49	−37·0	49	−37·0	24	−47·0	48	−37·0
67½	−33·0	73	−32·0	48	−37·5	74	−32·0
117½	−27·5	102	−29·0	72	−32·0	96	−30·0
173	−24·5	126	−27·0	98	−29·5	125	−27·0
242	−22·0	146	−25·5	120	−27·5	147	−25·5
		198	−24·0	146	−25·5	198	−23·5
		240	−22·0	175	−24·5	242	−22·0
				218	−23·0		

5		6		7		8	
Ox serum pseudoglobulin. 2 % in $N/4$ NaOH		Cow serum euglobulin. 2 % in $N/4$ NaOH		Ox serum euglobulin. 2 % in $N/4$ NaOH		Cow colostrum euglobulin. 2 % in $N/4$ NaOH	
1½	−75·5°	2	−74·5°	1½	−76·0°	2	−74·0°
24	−55·0	7	−66·5	24	−55·0	6	−67·0
48	−48·5	24	−54·5	50	−48·0	25	−54·0
72	−45·0	48	−48·0	72	−45·5	50	−48·0
96	−43·0	78	−44·5	96	−42·0	73	−45·0
144	−38·0	97	−42·5	144	−38·0	96	−42·0
189	−35·0	145	−38·0	197	−34·5	146	−38·0
240	−33·5	192	−35·0	240	−33·5	196	−35·0
		240	−34·0			242	−33·0

Comments on Table I.

In order to interpret more easily the different racemisation data, it is advantageous to represent the course of the racemisations graphically. Only one such graphical representation is given here for the pseudoglobulins (Diagram I). From a study of it, the following general features may be noted. The initial specific rotation of a 2 % pseudoglobulin solution in alkali is about − 80°; this factor diminishes rapidly in the first stages of the racemisation and ultimately attains a practically constant value of about − 22°. The curves illustrate clearly the different effects of $N/2$ and $N/4$ alkali. Though the strength of the alkali does not appear to affect appreciably the initial rotations of the solutions, yet the rate of fall of rotation is slower in the weaker alkali.

1. *Comparison of cow and ox serum globulins.*

The figures obtained for the euglobulin and pseudoglobulin of cow and ox serum demonstrate the identical optical behaviour displayed by these proteins not only in $N/2$ but also in $N/4$ NaOH. Such a result is of course in keeping with the presumed structural identity of corresponding proteins from different members of the same species, irrespective of sex. The determinations, however, afforded a check not only on the correctness of the method of working, but also on the chemical individuality of serum globulin prepared from different sources.

2. *Comparison of euglobulin and pseudoglobulin.*

The figures in Table I and Diagram I show that euglobulin and pseudoglobulin, whether from serum or from colostrum, display the same optical behaviour in $N/2$ as well as in $N/4$ alkali. This result, which harmonises with the assumption of the structural identity of euglobulin and pseudoglobulin, is confirmatory of the work of Miss Chick [1914], Hartley [1914], Crowther and Raistrick [1916], Dudley and Woodman [1918]. If, as Miss Chick postulated, the euglobulin is associated with traces of a lipoid substance, then the latter does not appear to have been present in sufficient amount in the samples investigated to affect their specific rotations in alkaline solutions to a measurable degree.

3. *Comparison of globulins of serum and colostrum.*

That the optical rotatory values of the globulins of serum and of colostrum are identical in $N/2$ and $N/4$ NaOH is shown by Diagram I, in which the data for the pseudoglobulins from the two sources have been plotted.

It will be seen that under each set of conditions, one curve can be drawn to satisfy equally the two sets of readings determined for the colostrum and the serum pseudoglobulin. Such slight discrepancies as may exist fall within the general error of experiment and this identity of optical behaviour may be taken as confirming the structural identity of the globulins of colostrum and of serum, a result which is in accord with the earlier chemical and biological work.

Racemisation of the albumins.

The figures obtained in the racemisation of the various albumins are given in Table II and Diagram II.

Table II. *Figures obtained in the racemisation of the various albumins. (The observations for the albumins of cow colostrum and ox serum in N/4 NaOH are given in Diagram II.)*

1 Cow serum albumin 2% in N/2 NaOH		2 Ox serum albumin 2% in N/2 NaOH		3 Cow colostrum albumin 2% in N/2 NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation	Time in hours	Specific rotation
1	−70·0°	1	−69·5°	1	−85·0°
24	−47·5	2	−67·0	2	−77·5
44	−39·5	7	−59·5	6	−68·5
73	−33·5	24½	−47·5	24	−54·5
96	−30·5	49	−38·5	48½	−44·5
120	−27·5	73	−34·0	73	−38·0
146	−25·0	98	−30·5	96½	−34·0
168	−22·5	121	−28·0	121½	−31·0
213	−19·5	149	−24·5	145	−28·5
240	−18·0	169	−23·0	168	−26·0
		192	−21·0	192	−24·0
		218	−19·0	217	−23·0
		242	−18·0	240	−22·0

4 Milk albumin 2 % in N/2 NaOH		5 Cow serum albumin 2 % in N/4 NaOH		6 Milk albumin 2 % in N/4 NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation	Time in hours	Specific rotation
1	−85·5°	1	−74·0°	1	−89·0°
4½	−70·5	25	−57·5	6	−79·5
24	−55·0	43	−53·0	23	−66·0
50	−44·0	67	−47·5	48	−58·5
72	−38·5	91	−44·5	68	−54·0
97	−34·0	125	−39·5	96	−49·0
120½	−31·0	144	−38·0	125	−45·5
149	−27·5	191	−34·0	140	−44·0
173	−26·0	261	−31·0	169	−41·5
193	−24·0			192	−38·5
240	−21·5			221	−37·0
				250	−35·5

Comments on Table II.

A comparison of the figures given in Tables I and II and Diagrams I and II demonstrates the distinct chemical individuality of the globulin and albumin fractions both in serum and in colostrum.

In carrying out the racemisation trials with the albumin preparations, it was noticed that in every case a small amount of sediment separated out in the first few hours from the alkaline solutions. This was readily removed by filtration, a perfectly clear solution remaining.

In all cases, the course of racemisation could be represented graphically by a perfectly smooth curve of the type already described. As with the globulins, the action of $N/4$ soda occasioned a slower rate of diminution of optical rotation than was the case when $N/2$ alkali was employed.

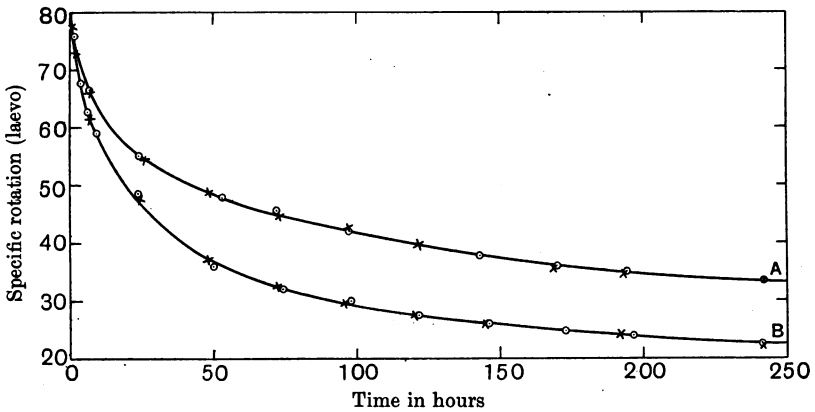

Diagram I. Showing identical optical behaviour of Cow Colostrum Pseudoglobulin [×] and Cow Serum Pseudoglobulin [⊙] in $N/2$ and $N/4$ NaOH.

Curve A. 2 % solutions of protein in $N/4$ NaOH.
Points denoted by × colostrum pseudoglobulin.
„ „ ⊙ serum pseudoglobulin.

Curve B. 2 % solutions of protein in $N/2$ NaOH.
Points denoted by × colostrum pseudoglobulin.
„ „ ⊙ serum pseudoglobulin.

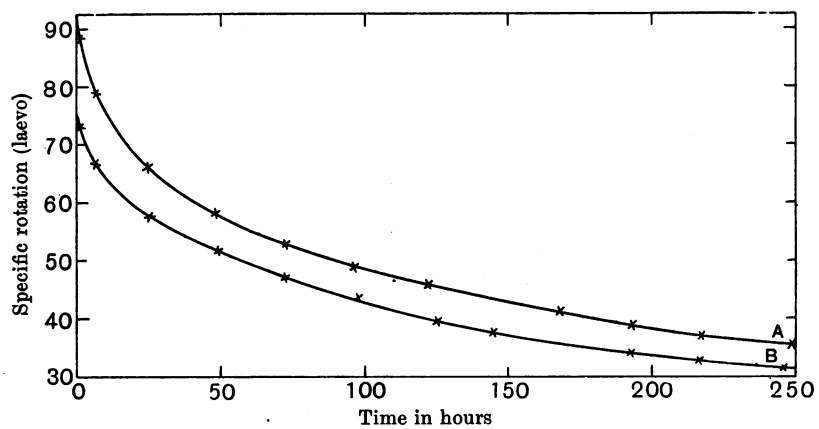

Diagram II. Showing different optical behaviour of Cow Colostrum Albumin (A) and Ox Serum Albumin (B) in $N/4$ NaOH.

Curve A. 2 % cow colostrum albumin in $N/4$ NaOH.
Curve B. 2 % ox serum albumin in $N/4$ NaOH.

1. *Comparison of cow and ox serum albumins.*

The results tabulated in Table II (1, 2, 5) and curve B, Diagram II, are conclusive as to the structural identity of the albumins of cow and ox serum.

2. *Comparison of the lactalbumins of colostrum and milk.*

The figures in columns 3, 4, and 6 of Table II and curve A, Diagram II show that colostrum and milk albumins display identical optical behaviour when incubated under comparable conditions with $N/2$ and $N/4$ soda. This is indicative of their structural identity and is in agreement with the results of biological tests and the work of Crowther and Raistrick [1916] on the van Slyke analysis of these proteins.

3. *Comparison of serum albumin and lactalbumin.*

The readings obtained in the racemisation of lactalbumin and serum albumin reveal marked differences between the optical behaviour of these proteins not only in $N/2$, but also in $N/4$ alkali. This difference is manifest in the initial rotations displayed in alkali, for whereas the specific rotation of serum albumin is initially about $-75°$, that of lactalbumin is about $-92°$ (taken from curves). A comparison of these data with the specific rotations for these proteins determined by Sebelien [1885] in aqueous solution shows a striking difference in the manner in which the nature of the solvent affects the rotation of the two proteins.

	Serum albumin	Lactalbumin
$[\alpha]_D$ in water	$-63°$	$-37°$
$[\alpha]_D$ in $N/4$ NaOH ...	$-75°$	$-92°$

These figures bring out very clearly the existence of structural dissimilarity between the two albumins. There can be little doubt that serum albumin and lactalbumin are two distinct proteins, though it is difficult to understand why the biological tests have failed to reveal this fact.

A study of Diagram II (which shows graphically the courses of racemisation in $N/4$ NaOH) reveals a remarkable parallelism between the curves of the two albumins.

The same feature is noticed if the curves in $N/2$ NaOH be drawn and compared. It may be that the structural differences existing between the two albumins are only of an inconsiderable character and that lactalbumin is actually formed from serum albumin as a result of comparatively slight changes brought about by the activity of the mammary gland. This speculation is suggestive of the possibility of a simple transformation of serum albumin into lactalbumin, or *vice-versa*.

Racemisation data of heat-coagulated pseudoglobulin.

A sample of colostrum pseudoglobulin was coagulated by raising the temperature of its solution in water gradually to 90°. The coagulated protein was filtered off, washed successively with alcohol and ether and dried in the steam oven. 2 % solutions in $N/2$ and $N/4$ NaOH gave the following readings on racemising at 37°.

In $N/2$ NaOH		In $N/4$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
1	−76·0°	2	−75·0°
4½	−66·0	24	−55·0
24	−47·5	48	−48·5
48	−38·0	72	−45·0
72	−32·5	120	−39·5
120	−27·0	165	−36·0
169	−24·5	216	−34·0
220	−22·5		

A comparison of these data with those plotted in Diagram I shows clearly that heat coagulation of the protein had not in any way affected its optical rotatory values in alkali.

Racemisation of samples of colostrum globulin prepared by absolutely different methods.

The writer had at his disposal, owing to the kindness of Drs. Crowther and Raistrick, a sample of colostrum pseudoglobulin which had been investigated by them in their comparative work already referred to. This had been prepared from colostrum by removing the caseinogen with saturated potash alum solution, and saturating the neutralised filtrate with anhydrous magnesium sulphate to obtain the globulin. The crude globulin was then purified by repeated salting out from solution with $MgSO_4$ and finally dialysed, the pseudoglobulin being subsequently thrown out of solution with alcohol. 2 % solutions of this preparation in $N/2$ and $N/4$ NaOH were investigated at 37° with the following results.

In $N/2$ NaOH		In $N/4$ NaOH	
Time in hours	Specific rotation	Time in hours	Specific rotation
2	−73·0°	2½	−73·0°
24	−47·5	24	−55·0
48	−37·5	49	−48·0
74	−31·5	73½	−44·5
96	−29·5	97	−41·5
144	−26·0	145	−38·0
196	−23·5	198	−35·0
240	−22·0	240	−33·5

A comparison of these figures with those enumerated in Table I and Diagram I shows that the samples of globulin prepared by two different methods displayed identical optical behaviour in $N/2$ and $N/4$ alkali. This result supports the view that globulin is a definite chemical individual, whose composition is unaffected by its method of preparation.

SUMMARY.

1. A simple optical method has been described whereby it is possible to establish identity or non-identity of related proteins.

2. Evidence of the identity of euglobulin and pseudoglobulin, whether from serum or colostrum, has been brought forward.

3. The identity of the globulins of cow and ox serum and of colostrum has been confirmed.

4. The albumin of milk has been shown to be identical with the albumin of colostrum.

5. Lactalbumin and serum albumin have been found to be two distinct proteins. From this, the conclusion is drawn that a distinct mammary synthesis is necessary for lactalbumin as well as for caseinogen.

6. The optical rotatory properties of pseudoglobulin in alkali have been shown to be unaffected by heat coagulation.

7. The corresponding proteins of cow and ox serum have been compared and found identical.

8. Evidence has been adduced in support of the view that globulin is a definite chemical individual, whose composition is unaffected by its mode of preparation.

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