The Calcium Binding of Human Serum Albumin in Health and Disease

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Martin & Perkins (1950), studying the calcium-binding capacity of human albumins prepared by a variety of methods, found good agreement between samples prepared from fresh material, provided due care had been taken in the fractionation. They drew attention to two exceptions. The one, a sample of albumin purified by repeated recrystallization with the aid of decanol, gave a low value; the other, a specimen of albumin which had been crystallized with the aid of mercury according to the method of Hughes (1947), gave a high value for calcium binding.

These findings raised the possibility that in normal human serum there are at least two species of albumin differing in their capacity to bind calcium.

In the present work albumins prepared from serum drawn from a number of normal healthy persons have been examined to discover to what extent the calcium binding varies from one individual to another and from one age group to another. In addition, albumins have been separated from the serum of a limited number of patients suffering from a variety of disease processes to discover whether or not disease may alter the calcium-binding capacity of the serum albumin.

A brief note of the observation on the calcium-binding capacity of serum albumin isolated from patients suffering from parathyroid tumours has already been reported by Martin & Perkins (1951). The results recorded in this paper are supplementary to those already published.

EXPERIMENTAL

Preparation of serum albumin. Blood was drawn without venous stasis, from fasting subjects at rest, into clean tubes without anticoagulant and allowed to clot. The fresh serum was removed and subjected to fractionation without delay by the cold methanol procedure of Pillemer & Hutchinson (1945). The albumin obtained in solution was dialysed against repeated changes of 0.15M-NaCl, the temperature never being allowed to rise above 2°. The final solution was transferred to clean screw-topped bottles and freeze dried. The dried material was then redissolved and the calcium binding studied by the method of equilibrium dialysis described by Martin & Perkins (1950).

Analytical procedures. Determinations of pH were made on all reconstituted samples using a glass electrode and a Cambridge potentiometer.

Protein nitrogen concentrations at each stage were estimated by the standard micro-Kjeldahl technique. Albumin/globulin ratios in the original serum were estimated independently, using Na_2SO_4 as the precipitating agent according to the method of Martin & Morris (1949).

The electrophoretic analyses were carried out in phosphate buffer, pH 8.0, ionic strength $I=0.2$ using the moving boundary technique of Tiselius (1939).

The ultracentrifugal analyses were carried out in the oil-driven centrifuge of Svedberg in phosphate buffer, pH 8.0, $I=0.2$.

Clinical diagnosis. The clinical diagnoses referred to, unless otherwise stated, have been confirmed by autopsy or operation. The clinical course of all patients has been followed to death or for not less than 2 years.

RESULTS

The serum albumin from twenty-nine normal persons whose ages ranged from 2 to 91 years was examined. The individual values are shown in the

Fig. 1. Values for calcium binding of albumin, isolated from twenty-nine individuals believed to be normal, plotted against age. ●, males; ○, females. Mean value, 4.75. Limits of deviation according to Student's distribution when $P=0.01$ are ± 0.9 . The dotted lines on the figures represent the range of these limits about the mean.

form of a graph in Fig. 1, together with the calculated mean and the limits of variation to be expected in 99 % of persons. The values for the five persons over

80 years show considerable scatter. While every care was taken to exclude organic disease, this was not easy in the oldest patients. In Table 1 are three sets of duplicate estimates, taken at yearly intervals from a single individual, whose detailed life history

Table 1. *Calcium-binding capacity of normal albumins*

(Albumins separated from the sera collected from two normal individuals on the dates shown. All analyses were carried out between pH 6.4 and 6.8 in 0.15 M-NaCl.)

Initial	Date	Bound Ca (mg./g. albumin N)
N.M.	28. xi. 50	4.4 4.8
N.M.	7. vi. 51	4.6 4.4
N.M.	20. x. 52	4.2 4.5
R.K.	10. vii. 51	5.0 4.3

was known. These values suggest that there are no gross variations from year to year; these and the duplicate analysis of a second subject show the variations in values to be expected from the inherent technical difficulties of the method.

Table 2 shows the analysis on eight patients suffering from chronic renal damage and five patients suffering from tumours of the parathyroid gland. The pre- and post-operative levels are shown in three of these patients, together with the post-operative levels on two other patients who have been under observation for 5 and 13 years, respectively. In four of the patients suffering from parathyroid tumours the post-operative percentage renal efficiency, as estimated by the urea clearance technique of Moller, McIntosh & Van Slyke (1928) is shown. A value of 75 % and upward was regarded as normal.

Analyses from six other patients who had been the object of careful clinical study are shown in Table 3. While there is no reason to doubt the diagnosis given in any one of these patients, they form a miscellaneous group, and the levels recorded may or may not be characteristic. They are presented to show that the calcium-binding capacity of serum albumin may be within normal limits in the presence of severe general metabolic disturbance.

Electrophoretic analyses of the albumin obtained by the methanol fractionation from two normal subjects, from two patients suffering from chronic renal damage and from one patient suffering from tumour of the parathyroid are shown in Fig. 2. Ultracentrifugal analyses of two albumins, the one

Table 2. *Calcium-binding capacities of twenty-two human serum albumins separated from thirteen patients in whom the clinical diagnosis was confirmed at autopsy or operation*

(All analyses were carried out between pH 6.3 and 6.9 in 0.15 M-NaCl.)

Patient and date of collection	Diagnosis	Albumin N in serum (g./l.)	N of sample in sac (g./l.)	Donnan coefficient calculated from chloride distribution	Calculated bound Ca inside sac (mg./l.)	Bound Ca (mg./g. albumin N)	Urea clearance Van Slyke method* (%)
B 15. iii. 50	Chronic glomerulonephritis	4.2	2.0	1.02	19.3	9.6	—
B 24. iii. 50		4.3	1.9	1.01	17.1	9.0	—
C 1. vi. 51		3.3	1.9	1.01	17.3	9.1	—
C 12. vii. 51		3.3	2.0	1.02	22.3	11.2	—
D 29. vi. 50		6.7	2.2	1.02	19.3	8.6	—
D 29. vi. 51		4.5	2.76	1.02	57.9	18.8	—
E 24. ix. 52		0.8	0.5	1.003	5.4	12.0	—
F 5. x. 51	Chronic pyelonephritis	3.2	2.75	1.02	32.9	11.9	—
G 29. ii. 52		4.6	1.9	1.01	13.3	7.3	—
H 15. vi. 50	Chronic glomerulonephritis	4.6	2.7	1.01	5.3	2.0	—
H 22. vi. 51		4.2	2.5	1.01	3.3	1.3	—
K 26. ii. 52		3.8	2.6	1.01	5.2	1.9	—
K 31. iii. 52		3.8	1.0	1.01	0.9	0.9	—
N 15. iii. 50	Parathyroid tumour pre-operative	7.4	1.6	1.02	10.7	6.7	—
O 12. vi. 51		6.4	2.4	1.01	21.8	9.0	—
P 17. v. 50		6.5	3.1	1.03	19.3	6.2	—
N 12. vii. 51	Parathyroid tumour post-operative	7.3	4.6	1.03	32.2	7.1	—
O 29. iii. 52		6.4	2.4	1.04	44.4	14.3	30
P 31. v. 50		6.5	3.0	1.04	14.8	4.9	—
P 28. v. 51		6.5	3.0	1.02	14.6	4.9	80
Q 11. v. 51		6.4	2.7	1.02	11.8	4.4	85
R 30. v. 50		4.8	2.7	1.03	17.0	6.3	50

* 75 % upward = normal clearance values by this method.

Table 3. *Calcium-binding capacities of six human serum albumins separated from six patients in whom the diagnosis was established by careful clinical assessment*

(All analyses were carried out between pH 6.4 and 6.6 in 0.15M-NaCl.)

Patient and date of collection	Diagnosis	Albumin N in serum (g./l.)	N of sample in sac (g./l.)	Donnan coefficient calculated from chloride distribution	Calculated bound Ca inside sac (mg./l.)	Bound Ca (mg./g. albumin N)
T.A. 20. vi. 50	Hypoparathyroid (post-operative)	6.4	3.0	1.03	5.4	1.8
C.H. 22. viii. 50	Hyperthyroid	6.0	3.1	1.02	14.3	4.6
G.R. 31. i. 51	Hypothyroid	5.8	2.9	1.02	12.8	4.4
R.O. 28. iii. 51	Acromegalic	5.8	3.1	1.03	14.5	4.6
L.A. 24. iv. 50	Multiple fractures	6.5	2.9	1.02	11.6	4.0
M.C. 2. ii. 50	Hypertensive	5.6	1.7	1.02	7.2	4.2

from a patient suffering from chronic renal damage and the other from a patient with a parathyroid

DISCUSSION

Pillemer & Hutchinson (1945) described a cold-methanol fractionation which separated almost all the serum albumin from the serum globulin at one step. They published a series of analyses which showed that the relation of serum albumin to serum globulin obtained by this method tallied closely with the values obtained by electrophoretic analysis.

Fig. 2. Electrophoretic analysis of five albumins separated from sera by methanol fractionation and dialysis against 0.15M-NaCl. The analyses were carried out at 1% (w/v) protein concentration in phosphate buffer, pH 8.0, $I=0.2$. (a), normal subject; (b), normal subject; (c), patient C, 12. vii. 51, nephritic; (d), patient D, 29. vi. 51, nephritic; (e), patient N, 15. iii. 50, parathyroid tumour.

tumour are shown in Fig. 3. No significant difference was demonstrated either in mobility or sedimentation constant of any of the samples.

Fig. 3. Ultracentrifugal analysis in phosphate buffer, pH 8.0, $I=0.2$. Field strength 270 000 g. Exposures 60 min. after reaching full speed. (a), patient D, 29. vi. 51; (b), patient P, 17. v. 50.

Martin & Morris (1949), in a study of methods of estimating the albumin/globulin ratio, confirmed Pillemer & Hutchinson's findings and showed that the concordance held good over a wide range of sera from a variety of conditions in which it is known that there is gross disturbance of albumin/globulin ratio.

Direct electrophoretic analysis of the material obtained by methanol fractionation from normal and pathological sera suggested that it was not less than 94% pure and that contaminating material was α -globulin. Further, calcium-binding studies on the material obtained by the more complicated

techniques of ether and ethanol fractionation gave values that agreed with those obtained by the methanol fractionation.

Leutscher (1940) observed that purified human albumin when examined electrophoretically in acetate buffer at pH 4.0 resolved into two components. His findings have been confirmed in a limited series, but are not presented here in order to avoid suggesting, in any way, that the two components observed are associated with variations in calcium binding.

The original observations on the calcium binding of serum albumin (Martin & Perkins, 1950) suggested that there were at least two varieties of the albumin molecule circulating in normal serum. If that be so, the ratio of the two albumins shows little variation through the normal life span and is materially affected neither by sex nor by the normal period of growth. Further, the limited analyses suggest that there is little variation in the individual from year to year, in conformation with the group survey.

It follows that the deviations from normal observed in patients suffering from advanced renal damage or from the effects of a parathyroid tumour are most probably the consequence of the disease process. Progressive renal damage is well known to be a complication of the activity of a parathyroid tumour (Albright, Aub & Bauer, 1934), while many pathologists have remarked on the hyperplasia of the parathyroids which may be associated with 'primary' renal disease (Gilmour & Martin, 1937; Gilmour, 1947).

The observation that two of the three subjects who continued to have a raised calcium-binding level after operation had continuing renal damage demonstrable by an entirely independent test, while two, who had normal post-operative calcium binding levels, had no demonstrable renal damage by the same test, stresses the close relationship between parathyroid activity and renal function.

Two patients with renal insufficiency yielded albumin with low calcium-binding levels. One of these was the subject of intensive clinical and pathological study over a period of years, and there is

some reason to suppose that, terminally, if not earlier in the course of her disease, she may have suffered from hypofunction of the parathyroid. One patient in the miscellaneous group is believed on clinical grounds to be suffering from parathyroid insufficiency. The calcium binding of that patient's albumin was low, by comparison with normals and with the other five patients listed in the miscellaneous group.

It should not be concluded that the observations described are peculiar to albumin or to bivalent ions. Calcium was chosen as the cation to be studied because of its biological importance, albumin because it could be fractionated with the minimum of disturbance and with reasonable purity. The data presented here are no more than a pattern which may have analogies in other disease processes, and with other proteins not yet sufficiently characterized to make reasonably accurate study possible.

SUMMARY

1. The calcium-binding capacity of sixty-two human albumins isolated from forty-eight persons has been examined.

2. The mean binding capacity of albumin separated from normal individuals calculated as mg. calcium/g. albumin nitrogen is 4.7 ± 0.9 ($P = 0.01$). There is no significant variation between the ages of 2 and 91 years nor with sex.

3. Of the ten patients suffering from chronic renal damage, eight had significantly raised levels of calcium binding. The level varied with the course of the disease.

4. The albumins from patients with tumours of the parathyroid had raised calcium binding prior to operation. Of the albumins studied from patients after operation three gave normal levels, these being from patients in whom there was no evidence of renal impairment.

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