the same time. The crystallization is complete within a few hours if the solution is kept at about 35° C.

(6) Recrystallization. This consists in re-precipitating the protein at pH 4.4 and 10° C. from a relatively dilute solution of the crystals in water and then proceeding as described in (5). The inhibitor can also be recrystallized from 20 per cent. alcohol at pH 5.0.

The details of the method of isolation are to be described in a future publication.

Further studies are being made on the mechanism of the trypsin-inhibiting action of the new crystalline protein and also on some of its physical and chemical properties.

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## ELECTROPHORETIC STUDIES IN RHEUMATIC FEVER[1]

RECENT summaries of available clinical and epidemiological knowledge concerning rheumatic fever[2, 3] and investigations of this disease[4] indicate that rheumatic fever may not be a specific infectious disease but may be the result of a response on the part of the host to external stimuli which is different from the response to the same stimuli of individuals who do not develop rheumatic fever. Attempts have been made to correlate the occurrence of rheumatic fever with the antibody response of the host to infection with the group A–β hemolytic streptococcus, and these have been recently summarized.[5]

In considering this matter in a broader fashion, it is known that the measurement of serum antibodies is one method of determining the reaction of the host to external stimuli. Since it is also known that serum antibodies are for the most part found in the gamma globulin fraction of human serum[6] it seemed desirable to determine whether the gamma globulin fraction of patients suffering from rheumatic fever was different from that of normal individuals.

Isolated reports of electrophoretic studies in rheumatic fever indicate abnormalities in the gamma globulin fraction. Longsworth, Shedlovsky and MacInnes[7] included three specimens from cases of rheumatic fever among those from a large number of diseases without specifying stage of activity, and reported the gamma-albumin ratio to be elevated in all three patients. Luetscher[8] performed a similar study on a number of diseases and reported that a specimen from each of two cases of acute rheumatic fever with rheumatic heart disease and congestive failure contained a greater than normal amount of gamma globulin expressed as grams per hundred milliliters of plasma. In addition he stated that a specimen from one "old case" of rheumatic heart disease showed a similar change but no quantitative result was reported for that case.

### METHOD OF STUDY

Electrophoretic patterns were obtained from the various sera in the Tiselius apparatus by using the "Schlieren-scanning method of Longsworth."[9, 7] Before electrophoresis the sera were diluted 1 : 4 or 1 : 6 with buffer solution of pH 7.6 containing 0.15 M. NaCl and 0.02 M. sodium phosphate and dialyzed against large volumes of this buffer at + 2° C. Measurements were made by a planimeter of the areas under the various peaks in the photographic record for computing the relative amounts of the various components present, using the patterns for the descending boundaries. The total protein content of the sera was determined by micro-kjeldahl nitrogen analysis.

### RESULTS OF THE STUDY

As indicated in Table 1, the ratios of gamma globulin to albumin, and gamma globulin to total protein in nine specimens of serum from eight patients in the acute stages of the disease are elevated above "normal"[10] without exception, although one of these, serum (line 8, table 1), is at the upper limit of normal. Electrophoretic analysis of the serum in three of the same patients during inactivity of the disease show continued elevation of these ratios at a lower level. Four specimens from three individuals not suffering

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TABLE 1

| Name | Diagnosis | | Drug therapy | Outcome | Total protein (grams per cent.) | Electrophoretic analysis of serum: Areas A | $a_1 + a_2$ | $\beta$ | $\gamma$ | $\frac{\gamma}{\text{Total}}$ per cent. | $\frac{\gamma}{A}$ | T.P. (gm/per cent.) $\times \frac{\gamma}{\text{Total}}$ | $\frac{a}{A}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Class* | | | | | | | | | | | |
| F.Q. | Acute R.F. R.H.D.–M.S.–M.I. | I–E | None* | Rec. | ... | 113 | 17 | 28 | 38 | 19.4 | 0.34 | ... | .15 |
| F.Q. | R.H.D.–M.S.–M.I. | I–A | None | Rec. | 7.50 | 123 | 17 | 26 | 31 | 15.7 | 0.25 | 1.17 | .14 |
| J.C. | Acute R.F. R.H.D.–M.I. | I–E | None* | Rec. | 7.62 | 76 | 30 | 28 | 53 | 28.4 | 0.70 | 2.16 | .39 |
| J.C. | R.H.D.–M.I. | I–A | None | Rec. | 7.37 | 113 | 14 | 20 | 33 | 18.3 | 0.29 | 1.35 | .12 |
| G.S. | Acute R.F. R.H.D.–M.I. | II–E | None* | Rec. | 8.50 | 119 | 18 | 28 | 54 | 24.8 | 0.45 | 2.12 | .15 |
| G.S. | R.H.D.–M.I. | II–B | None | Rec. | 7.25 | 109 | 10 | 21 | 42 | 23.0 | 0.39 | 1.67 | .09 |
| R.S. | Acute R.F. R.H.D.–M.S.–M.I. | II–E | None* | ? | ... | 74 | 20 | 33 | 52 | 29.0 | 0.70 | ... | .27 |
| R.S. | Acute R.F. R.H.D.–M.S.–M.I. | II–E | Salicylates | ? | 7.25 | 76 | 31.5† | | 17.5 | 14.0 | 0.23 | 1.02 | .. |
| A.L. | Acute R.F. R.H.D.–M.I. | I–E | None | Rec. | 7.75 | 75 | 24 | 25 | 51 | 29.3 | 0.68 | 2.27 | .32 |
| J.M. | Acute R.F. R.H.D.–M.I. | II–E | Small doses salicylates | Rec. | 8.12 | 98 | 29 | 30 | 45 | 22.2 | 0.46 | 1.80 | .30 |
| S.C. | Acute R.F. R.H.D.–M.S.–M.I. | IV–E | Massive doses of salicylates and treatment for congestive failure | Died 2 days after sample was taken | 7.25 | 61 | 41 | 24 | 44 | 25.9 | 0.72 | 1.88 | .67 |
| V.V. | Acute R.F. (Sed rate) R.H.D.–M.S.–M.I. | II–E | None | Rec. | 7.00 | 118 | 13 | 24 | 33 | 17.6 | 0.28 | 1.23 | .11 |
| D.R. | Control | | .... | .... | 7.75 | 131 | 17 | 38 | 26 | 12.0 | 0.20 | 0.93 | .13 |
| D.R. | Control | | .... | .... | 7.50 | 120 | 46† | | 23 | 12.2 | 0.19 | 0.92 | .. |
| K.J.T. | Control | | .... | .... | 7.37 | 78 | 7 | 28 | 14 | 11.0 | 0.18 | 0.81 | .09 |
| B.D. | Control | | .... | .... | 7.37 | 68 | 41† | | 25 | 18.6 | 0.37 | 1.37 | .. |

* Salicylate therapy began after taking of specimens.
† $a_1 + a_2 + \beta$.

from rheumatic fever analyzed in the same electrophoretic cell reveal ratios similar to those reported for normals in the literature in three, and the serum in one (line 16, table 1), was found to be abnormal. The alpha globulin-albumin ratios reported as being increased in cases of febrile disease[7] are less consistently elevated in the specimens analyzed in this study.

It is appreciated that the reported results are only suggestive and much further work along these lines remains to be done. The reported changes may be just another non-specific manifestation of rheumatic fever. However, this method of study offers a quantitative approach not heretofore available in the study of the mechanism of this obscure disease, since it is possible to study in quantitative fashion a constituent of the blood which is found in increased amounts in rheumatic fever.

### Summary and Conclusions

Electrophoretic analysis of specimens of the blood serum of a small number of cases of rheumatic fever during the "active" and "inactive" stages of the disease show consistent increases above "normal" in the gamma globulin fraction of the serum.

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## URONIC ACIDS IN ANIMAL BODIES

It is known generally that quantitative methods for estimating glucuronic acid in biological materials are not specific but may include other uronic acids. In spite of this, much of the literature on the subject ignores the possibility that galacturonic acid and its salts may also be present in animal tissues and fluids. There is perhaps justification, in the case of the carnivore, for considering the uronic acid as glucuronic. This assumption is not justified for the herbivore where it is known that the diet is rich in polygalacturonic compounds in the form of pectic substances. (Pectin "consists chiefly of partially