further investigation may not produce alternate procedures equally satisfactory.

*Results.* Table III presents a series of analyses on blood serum taken under oil, compared to results on the manometric method of Van Slyke *et al.*(6). The mean result is 100.05% of the Van Slyke value with a standard deviation of ±1.70 and a standard error of 0.31. Thus, it is evident that the present method yields a fairly high degree of precision even though carried out on one-tenth of the amounts that the Van Slyke method employs.

*Summary.* 1. A direct acidimetric titration method is presented for the estimation of serum carbonate in small volumes of serum or plasma. Serum bicarbonate is precipitated as the barium salt by the addition of barium hydroxide, the precipitated bicarbonate is dissolved in hot boric acid, and the $Ba(H_2BO_3)_2$ in solution is titrated with standard acid to the pH of a pure solution of boric acid with the aid of an ultramicro burette. The titration represents the bicarbonate in equivalents. 2. Various critical points in the method were investigated and are discussed. The probable existence in serum of a protein bound carbonate is indicated. There is close agreement between the values obtained in this method on 0.1 ml of serum and the results obtained by the manometric procedure of Van Slyke, employing 1.0 ml of serum.

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## Comparative Studies of the β Disturbance of Electrophoretic Patterns in Disease. (19468)

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In electrophoretic patterns of the descending boundary of plasma or serum obtained by the Schlierin scanning technic, the β globulin component often contains a sharp spike extending upward. In an electrophoretic study of the serum from poliomyelitis patients, Kelley and coworkers observed a definite decrease in the length of this β spike(1,2).

In recent studies on poliomyelitis in this laboratory(3), the decrease of the length of the β spike in plasma and serum patterns was compared with those from normal individuals. Since the results, especially those from normal individuals, did not agree with those reported by Kelley *et al.*(2), it was decided to compare the length of the β spike of electrophoretic patterns of plasma and serum from several diseases.

TABLE I. The Beta Spike of Electrophoretic Patterns of Plasma.

| Disease | No. of samples | Ratio of beta spike to albumin peak, % | Change in length of beta spike, % None or slight | Marked |
|---|---|---|---|---|
| Normals—children | 77 | 42.4 | 39 | 61 |
| " —adults | 37 | 46.2 | 45.9 | 54.1 |
| Poliomyelitis | 134 | 36.4 | 37.3 | 62.7 |
| Rheumatic fever | 224 | 41.3 | 36.2 | 63.8 |
| Liver disease | 142 | 34.2 | 36.6 | 63.4 |
| Diabetes | 202 | 31.4 | 16.3 | 83.7 |
| Arthritis | 134 | 42 | 44 | 56 |
| Arthritis before ACTH or cortisone | 19 | 24.5 | 15.8 | 84.2 |
| Arthritis after ACTH or cortisone | 24 | 51.1 | 41.6 | 58.4 |

*Experimental.* The plasma and serum samples were obtained from normal children, normal adults and from patients with various diseases. These samples were diluted with 3 volumes of a barbiturate buffer, pH 8.6, ionic strength 0.1, and dialyzed at 2 to 6°C for 3 days with daily change of buffer. Electrophoresis was carried out for 120 minutes in the Longsworth modification of the Tiselius apparatus using the analytical cell. The electrophoretic pattern of the descending boundary was used for the measurement of the length of the β spike. Complete electrophoretic data on the plasma and serum patterns from many of the subjects in this paper have already been reported in previous publications from this laboratory.

Kelley and coworkers graded their patterns as follows: 0 or no change from the normal, where the β spike was long and narrow and extended upward to the full length of the albumin peak. 1+ indicated a shortening and broadening of the β spike with the length equal to about 2/3 that of the albumin peak. 2+ indicated a marked decrease of the β spike to approximately 1/3 the length of the albumin peak. 3+ indicated complete absence of the β spike.

An attempt was made to devise a more quantitative comparison of the β spikes of the various electrophoretic patterns. For this purpose, the albumin peak and β spike of each pattern were measured. A ratio representing the length of the β spike expressed as a percentage of the length of the albumin peak was then calculated. Values obtained in this fashion were compared with the Kelley system of rating. Since a range of values was necessary for accurate comparison, these are also shown below.

0 = 100% (of the length of albumin peak) or a range of 83.3 to 100%
+1 = 66.6% or range of 50 to 83.3%
+2 = 33.3% or range of 16.6 to 50.0%
+3 = 0 or range of 0 to 16.6%
No or slight change, 50 to 100%
Marked change, 0 to 50%

*Results.* Employing the rating system outlined above, the length of the β spikes of approximately 1100 plasma and 500 serum patterns were examined. Table I represents the results obtained from the plasma samples. A comparison of the ratio of the length of the β spike to the length of the albumin peak of these patterns shows that plasma from normal children or adults exhibits a ratio similar to those from patients with various diseases. Although the average length of the β spike in disease was shorter than that from normal individuals, this difference was not as marked as that in the serum samples reported by Kelley and coworkers. An interesting observation was the marked increase in the ratio observed in a small group of arthritis patients after treatment with ACTH or cortisone.

Kelley *et al.* designated a change in the length of the β spike as "no or slight" if the pattern was judged 0 or +1. They called a change "marked" if the pattern was judged +2 or +3. For comparative purposes, our patterns were judged as previously described

TABLE II. The Beta Spike of Poliomyelitis Plasma Patterns.

| Patient classification | No. of samples | Ratio of beta spike to albumin peak, % | Change in length of beta spike, % None or slight | Marked |
|---|---|---|---|---|
| All patients | 134 | 36.4 | 37.3 | 62.7 |
| Spino-bulbar | 22 | 37.8 | 40.1 | 59.1 |
| Spino-bulbar, died | 13 | 27.4 | 30.8 | 69.2 |
| With paralysis | 17 | 38.7 | 35.3 | 64.7 |
| With weakness | 23 | 37.8 | 30.5 | 69.5 |
| Abortive or non-paralytic | 72 | 35.7 | 37.5 | 62.5 |

TABLE III. The Beta Spike of Electrophoretic Patterns of Serum.

| Disease | No. of samples | Ratio of beta spike to albumin peak, % | Change in length of beta spike, % None or slight | Marked |
|---|---|---|---|---|
| Normals—children | 19 | 29.3 | 21.1 | 78.9 |
| " " | 20 | — | 80 | 20 |
| Kelley *et al.* | | | | |
| Normals—adults | 24 | 36.2 | 29.2 | 70.8 |
| Poliomyelitis | 47 | 38.3 | 34.1 | 65.9 |
| " Kelley *et al.* | 142 | — | 50 | 50 |
| Rheumatic fever | 132 | 35.7 | 24.2 | 75.8 |
| Liver disease | 80 | 21.6 | 20 | 80 |
| Diabetes | 54 | 27.6 | 18.5 | 81.5 |
| Arthritis | 72 | 48.2 | 52.8 | 47.2 |

and divided into those showing marked change and those showing no or slight change. These values are shown in Table I and exhibit marked similarity, with the exception of patterns from diabetes and the small group of arthritis patients before treatment with ACTH or cortisone.

A similar study of the β spike in plasma samples from poliomyelitis is shown in Table II. The classification of patients is similar to that previously reported from this laboratory(3). Kelley and coworkers were unable to observe a correlation between the change in the length of the β spike and the age and sex of the patient, the extent of paralysis, the occurrence of bulbar symptoms or the temperature and pulse of the patient at the time the blood sample was obtained. The only marked difference in the ratio of the β spike to the albumin peak was exhibited by the patients with spino-bulbar poliomyelitis that died. Also, this group and the group of patients that exhibited weakness had the highest percentage of marked changes in the β spike.

Table III presents a study of the changes in the length of the β spike in serum samples. The ratio of the β spike to the albumin peak in these patterns shows similar values for normal individuals and patients with various diseases. The difference in our results and those obtained by Kelley *et al.* is clearly shown in the last 2 columns in Table III. Kelley's value for normal children shows a high percentage of patterns with long β spikes which would result in a very high ratio of the β spike to the albumin peak. Our values for normal children and adults almost reversed those of Kelley *et al.* The changes in length of the β spike shown by our patients with disease are, in general, more marked than those reported by Kelley and coworkers in poliomyelitis. Patients with arthritis were the only ones that exhibited patterns similar to those of Kelley *et al.*

*Discussion.* The essential difference between our results and those reported by Kelley and coworkers is the length of the β spike in electrophoretic patterns obtained from normal individuals. Apparently a β spike which extends upward to the full length of the albumin peak is not characteristic of a normal

individual or of a patient with disease. The appearance and length of the $\beta$ spike depends somewhat on the dietary state of an individual, the amount of fat present in the blood, and the time of withdrawal of the blood sample. It is interesting to note that in the classical electrophoretic patterns reported by Longsworth and coworkers the $\beta$ spike was evident in most patterns and was long and narrow(4). On the other hand, patterns reproduced in more recent publications very often show a very short $\beta$ spike or in some cases the complete absence of this spike. It is also interesting to observe that in the present investigation plasma and serum samples from normal children exhibit shorter $\beta$ spikes than those from normal adults.

Before drawing any definite conclusions as to the cause of the slight differences between the length of the $\beta$ spike in patterns from normal individuals or those from patients with disease, the origin of the $\beta$ spike must be determined. We plan to extend this investigation to include a study of the origin of the $\beta$ spike in electrophoretic patterns of plasma and serum.

*Summary.* 1. Electrophoretic patterns of approximately 1100 plasma and 500 serum samples have been examined for changes in the length of the $\beta$ spike. These samples were obtained from normal children and adults and from patients with poliomyelitis, diabetes, rheumatic fever, arthritis, and liver disease. 2. Minor changes occurred in the average length of the $\beta$ spike in electrophoretic patterns from plasma and serum when patients with disease were compared with normal individuals. These changes were also observed when patterns from patients with one disease were compared to patterns from other diseases. These changes were not marked enough to differentiate between diseases or between normal individuals and those with disease.

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## Effect of Lyxoflavin on Growth of Baby Pigs Fed a Synthetic Diet. (19469)

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In 1949 Pallares and Garza(1) isolated from human heart myocardium a pentoseflavin which they identified as l-lyxoflavin. The significance of the presence of lyxoflavin in human heart muscle was not discussed by these investigators. However, Emerson and Folkers(2) have suggested the possibility that lyxoflavin may be a new member of the vit. B complex. Lyxoflavin differs from riboflavin only in the configuration of the groups about carbon 4 of the pentose side chain.

The present experiments were designed to study the effect on growth of feeding lyxoflavin to baby pigs on a "synthetic milk" ration containing all of the known nutrients.

*Experimental.* Baby pigs from one to 2 days of age were used as experimental animals. The technic of feeding and care of the animals has been described by Johnson *et al.*(3). The composition of the basal rations used in these experiments is given in Table I. In addition

TABLE I. Composition of Basal Diets.

| | Casein diet, % | Alpha-protein diet, % |
|---|---|---|
| Casein (vit.-free) | 25 | — |
| Alpha-protein | — | 24.6 |
| DL-Methionine | — | .4 |
| Sucrose | 68 | 68 |
| Cottonseed oil | 1 | 1 |
| Minerals | 6 | 6 |

The above materials were made into a "milk" containing 19.5% solids.