The effects on glucose tolerance curve are shown in Fig. 1 (staphylococcus infection) and 2 (intradermal staphylococcus toxin). In both cases there was evidence of a decrease in carbohydrate tolerance with a marked increase in the peak of the curve and a delayed return to fasting levels. This was quite marked following the second injection of toxin, the curve here presenting many points of similarity to that seen in mild diabetes.

*Discussion.* It is evident that repeated injections of *Staphylococcus aureus* or staphylococcus toxin accentuates and makes permanent the decrease in glucose tolerance which had been observed following a single lot of injections. The exact cause of this decreased tolerance has not been determined. Some preliminary experiments on rats, however, have shown that repeated injections of staphylococcus toxin may lower the insulin content of the pancreas.[2]

The effect of a greater number of injections of both organisms and toxin are being studied to determine whether or not a more severe glucose intolerance, simulating diabetes, may be produced.

*Summary.* Two massive doses of *Staphylococcus aureus* or staphylococcus toxin produced a definite and permanent decrease in carbohydrate tolerance, with glucose tolerance curves similar to those seen in mild diabetes.

13470

## Electrophoretic Identification of Antibody to Tuberculin Protein.*

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It has been shown[1, 2] that rabbits can be readily sensitized to tuberculin protein by repeated injections of the antigenic form of the protein. The sensitization can be demonstrated by means of the precipitin test,[1] the Arthus reaction,[2] anaphylaxis and the uterine strip contraction.[3] Obviously antibodies to the tuberculin protein

2 Nicholson, T. F., and Haist, R. E., unpublished results of work done in the Departments of Pathological Chemistry and Physiological Hygiene, University of Toronto.

* Aided by a grant from the Committee on Medical Research of the National Tuberculosis Association.

1 Seibert, F. B., *Am. Rev. Tuberc.*, 1930, **21**, 370.

2 Seibert, F. B., *J. Infect. Dis.*, 1932, **51**, 383.

3 Lewis, J. H., and Seibert, F. B., *J. Immunol.*, 1931, **20**, 201.

are present in the blood stream, as is the case with other protein sensitizations.

Antibodies to the pneumococcus[4-7] and to tetanus antitoxin[8] have been identified in the respective antisera not only by the well-known immunological technics but also recently by means of the Tiselius electrophoresis apparatus.[9] They have been shown to exist as components with mobilities similar to or close to that of normal γ-globulin.

It seemed worth while to attempt the identification of the antibody to tuberculin protein by this method. The following experiments approached the problem in two different ways. (1) Changes in the blood sera of rabbits were noted during sensitization with the tuberculin protein. Table I shows that in seven such sensitized rabbits the percentage of the α-globulin fraction was raised, while the albumin decreased. The ranges and average percentages of the serum proteins of 12 normal rabbits are given for comparison. (2) Immune

TABLE I.

| Serum | Total conc. % | % of total as | | | | % α-globulin removed |
|---|---|---|---|---|---|---|
| | | Albumin | Globulins α | Globulins β | Globulins γ | |
| Avg of 12 normals | 5.4 | 76.0 | 1.1 | 10.8 | 12.2 | |
| Range | (4.5-6.2) | (72.2-81.5) | (0-2.5) | (8.4-12.9) | (9.4-14.3) | |
| No. 7 | 5.4 | 61.0 | 6.7 | 11.6 | 20.7 | |
| No. 82 | 5.8 | 67.7 | 8.6 | 12.2 | 11.5 | |
| No. 84 | 7.2 | 70.7 | 6.9 | 10.4 | 12.0 | |
| No. 2083 | 6.5 | 58.0 | 7.9 | 14.1 | 20.1 | |
| No. 140 | 6.5 | 70.6 | 5.2 | 10.2 | 13.9 | |
| No. 140 specific precipitate removed | 6.6 | 71.5 | 3.6 | 10.5 | 14.5 | 30.7 |
| No. 3 | 5.8 | 59.8 | 8.5 | 14.6 | 17.1 | |
| No. 3 " " | 6.2 | 60.4 | 7.2 | 14.9 | 17.6 | 15.3 |
| No. 196 | 5.0 | 64.0 | 8.7 | 12.6 | 14.8 | |
| No. 196 " " | 4.5 | 63.5 | 7.8 | 13.9 | 14.4 | 10.3 |

All electrophoretic analyses were made on sera diluted 1:4 in phosphate buffer, pH 7.7, $\mu = 0.1$, with a potential gradient of 6.6 to 7.1 volts/cm. Each figure represents the average concentration calculated from four Svensson-Philpot curves. All antisera gave precipitin titers of 1:100,000 or 1:200,000 with the specific antigen.

4 Tiselius, A., and Kabat, Elven A., *J. Exp. Med.*, 1939, **69**, 119.

5 Blix, G., *Z. ges. exp. Med.*, 1939, **105**, 595.

6 Moore, D. H., van der Scheer, J., and Wyckoff, R. W., *J. Immunol.*, 1940, **38**, 221.

7 van der Scheer, J., Lagsdin, J. B., and Wyckoff, R. W., *J. Immunol.*, 1941, **41**, 209.

8 van der Scheer, J., and Wyckoff, R. W., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 427.

9 Tiselius, A., *Trans. Faraday Soc.*, 1937, **33**, 524.

sera (Nos. 140, 3, and 196) which showed high globulin percentages were precipitated with the homologous antigens at the equivalence point, the precipitates centrifuged off and then the remaining sera were studied in electrophoresis under conditions identical to those employed with the original serum, in order to determine in what fraction a loss might have occurred. The table shows that the $\alpha$-globulin fraction was partially decreased in these experiments.

Therefore, it would appear that the antibody to the tuberculin protein may exist in immune sera as a component with a mobility similar to or very close to that of $\alpha$-globulin, which in the normal rabbit is extremely small in amount.

It is probable that this antibody is not important as a defense mechanism against the disease tuberculosis since it was shown earlier[10] that guinea pigs rendered highly sensitive to the tuberculin protein so that they exhibited typical Arthus reactions and high precipitin titers, were not protected against infection. They were, in fact, more susceptible. Further attempts are being made to detect other antibodies with possibly greater significance in immunity.

An unknown component, designated as $\chi$-component, appeared in

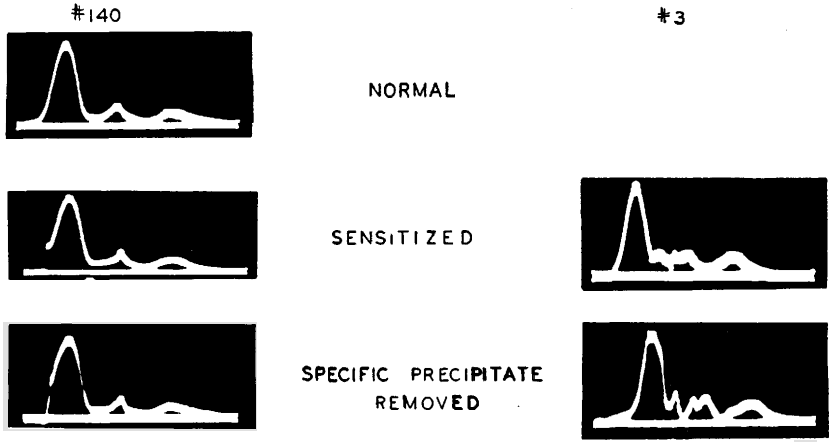


FIG. 1.

Electrophoretic (Svensson-Philpot) diagrams of 2 immune sera; descending side. No. 140 shows the picture in the normal serum where practically no $\alpha$-globulin is visible; serum after sensitization, where the increase in $\alpha$-globulin is seen as a definite rise from the base-line and the fast component appears as a break in the leading side of the albumin curve; serum after specific precipitate is removed, showing the return of the $\alpha$-curve to the base-line. Serum No. 3 shows a more significant rise in the $\alpha$-globulin, but the decrease of 15% in $\alpha$-globulin when the specific precipitate is removed, is better noted by accurate measurement than by observation.

[10] Seibert, F. B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1274.

most sera during sensitization. It had a mobility slightly greater than albumin and can be seen in the diagrams for rabbit No. 140. In another communication[11] it has been suggested that this may be due to the presence of tuberculin protein in the serum.

An increase in the α-globulin component occurred also in a rabbit sensitized in a similar manner with crystalline ovalbumin, and may, therefore, possibly be a general phenomenon in protein sensitization.

*Conclusion.* Antibody to tuberculin protein has been identified in serum as a component with an electrophoretic mobility close to α-globulin. This component increases in sera following sensitization to the protein, and is removed in part with the specific precipitate produced by adding antigen to the immune serum.

## 13471

## Urinary Excretion of Pantothenic Acid by Normal Individuals.

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Excretion studies have frequently been employed in assessing the dietary requirement for certain nutritive essentials. It is axiomatic that individuals consuming diets deficient in a particular nutritive essential will excrete less of the material in question than those subsisting on an adequate or optimum regimen. The establishment of normal excretion values is, therefore, a requisite for the application of such a method in evaluating the adequacy of diets.

Pantothenic acid is a dietary essential for a variety of species. Evidence has been presented by Spies, *et al.*,[1] that it is essential in human nutrition. Pelczar and Porter,[2] using a method based on the essential nature of pantothenic acid for *Proteus morganii,* found that the pantothenic acid content of 24-hour urine specimens from 9 persons ranged from 1.46 mg to 6.79 mg and averaged 3.81 mg. Pearson,[3] using the method of Pennington, Snell, and Williams,[4]

11 Seibert, F. B., and Nelson, J. W., *J. Biol. Chem.,* in press.

1 Spies, T. D., Stanbery, S. R., Williams, R. J., Jukes, T. H., and Babcock, S. H., *J. Am. Med. Assn.,* 1940, **115**, 523.

2 Pelczar, M. J., and Porter, J. R., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 3.

3 Pearson, P. B., *Am. J. Physiol.,* 1941, **135**, 69.

4 Pennington, D., Snell, E. E., and Williams, R. J., *J. Biol. Chem.,* 1940, **135**, 213.