antigen as now prepared it should be possible for any technician, who can properly standardize complement and perform satisfactory Wassermann tests, to obtain accurate and reliable results that will satisfy clinicians, with the complement fixation test of gonorrheal infection.

## 6180

## A "Lipoid" Extract of Spleen that Prevents Bartonella Muris Anemia in Splenectomized Albino Rats.

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The authors[1] have demonstrated that minute splenic autoplastic transplants made 7 weeks prior to splenectomy protect a large percentage of splenectomized rats from *Bartonella muris* anemia. A comparative histological study of the transplants of protected and unprotected rats revealed a regeneration of the pulp cells in the protected rats and an exhaustion destruction of the pulp in the unprotected rats. This supported the hypothesis that the reticular and endothelial cells of the pulp of the spleen possess some internal secretory substance. The parabiotic experiments of Lauda[2] further support the internal secretory action of the spleen. He found that rats joined by parabiosis are protected if the spleen of only one animal is removed.

Many investigators have tried and failed to demonstrate some substance in the spleen which would replace the spleen in protecting adult splenectomized rats against *Bartonella muris* anemia. We have made many attempts during the past three years to obtain such an extract. Lipoid extracts of the spleen were prepared which possess the property of protecting splenectomized adult albino rats against *Bartonella muris* anemia in a large percentage of instances. Since the anemia in the male is more severe, only male rats were used in testing these extracts. Of 440 male rats of carrier stock used for studies of *Bartonella muris* anemia during the past 3 years, not a single rat failed to develop *Bartonella muris* anemia following splenectomy.

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[2] Lauda, E., and Flaum, E., *Z. ges. exp. Med.*, 1930, **73**, 293.

*Preparation of the extract.* The spleen extract was prepared in a manner similar to Hartman's method of extraction of the suprarenal cortical hormone. Ox spleen freshly obtained from the slaughter house is repeatedly extracted with peroxide-free ether in the dark in an atmosphere of $CO_2$. The ether extractions are evaporated *in vacuo* at 15-20°. This residue is thoroughly extracted with warm 95% alcohol, then chilled to —10 degrees and filtered. The filtrate is evaporated to one-third the volume and again chilled and filtered and evaporated to dryness. The residue is taken up in a small volume of ether, filtered and dried *in vacuo*. Further extraction with alcohol may be necessary with subsequent chilling and filtering. The final residue is a small amount of thick gummy oil which is difficult to dry. It is thoroughly mixed with the desired quantity of distilled water and filtered through a Zeiss filter, brought up to isotonicity and preserved with 0.1% benzoic acid. One cc. of extract is equivalent to 100 gm. of spleen.

*Experimental data.* Twenty-nine albino male rats of carrier stock were tested with the lipoid extract of spleen made in this manner. Of these 8 were 6-8 weeks of age and 21 were 3-5 months old. The extract was administered twice daily intraperitoneally in amounts of 0.5 cc. The injections were started 24 hours prior to splenectomy or on the day of operation. The hemoglobin estimation with the Dare hemoglobinometer, the red blood cell count and smears were made daily. It was necessary that protection be observed for a period of one month to eliminate a delayed appearance in the anemia following splenectomy. All the rats were of heavily infected stock raised in our laboratory and used for studies of this anemia during a period of several years.

Of the first group of immature rats complete protection against *Bartonella muris* anemia was obtained in 3 of 8 instances. Of the second group of mature rats 14 of 21 were completely protected. Of 40 male splenectomized rats injected daily with physiological salt solution, all developed *Bartonella muris* anemia.

TABLE I.
Protective action of a "lipoid" extract of spleen against *Bartonella muris* anemia in male splenectomized rats of carrier stock.

| No. Rats | Age | Completely Protected | Unprotected | % Protected |
|---|---|---|---|---|
| **Treated** | | | | |
| 8 | 6-8 weeks | 3 | 5 | 37 |
| 21 | 3-5 months | 14 | 7 | 66 |
| **Controls 0.5 cc. saline twice daily** | | | | |
| 40 | 3-5 months | 0 | 40 | 0 |

Rats of carrier stock with the spleen intact, between the ages of 6 and 8 weeks, suffer from a severe infection of *Bartonella muris* with little or no anemia. This is manifested by the occasional appearance of *Bartonella muris* bodies in the blood cells and the marked hyperplasia and congestion of the spleen. Protection in such instances against anemia following splenectomy by an extract of spleen is obviously, therefore less effective. In the adult, the infection is latent and the spleen shows little evidence of hyperplasia and congestion. In these rats protection was obtained in a large percentage of instances.

A "lipoid" extract of the spleen was prepared which protects adult albino rats of carrier stock in a large percentage of instances against *Bartonella muris* anemia following splenectomy. One cc. of extract (corresponding to 100 gm. of fresh spleen) is injected daily intraperitoneally in divided doses for a month. It is suggested that the extract contains a specific hormonal substance.

## 6181

## Protective Action of Copper and Iron against Bartonella Muris Anemia.*

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Steenbock and his coworkers,[1, 2, 3, 4, 5] Beard and Myers[6, 7, 8, 9, 10, 11]

* The copper and iron were obtained through the courtesy of the Myron L. Walker Company, Mt. Vernon, N. Y.

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