influence the composition of the collected samples. It follows that this factor must be accounted for if the method is to be used to calculate statistical distribution of nephron lengths and total volume of the nephron plus collecting system.

Finally, these results indicate that the actual localization of tubular activity by the stop flow method is perhaps less precise than realized. In particular, any quantitative conclusion that does not account for fluid movement and consequent introduction of new filtrate must be interpreted with caution.

*Summary.* The movement of intratubular fluid during stop flow has been investigated by injecting 2 or more filtration indicators at different intervals during period of ureteral occlusion. Appearance of these indicators in different samples collected upon release of the occlusion demonstrates that appreciable fluid movement takes place during stop flow.

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## Differences between Antigenic Specificity of Nonsoluble Particles and Soluble Extracts Prepared from Brucellae by Disintegration.* (24952)

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Olitzki and Sulitzeanu(1,2,3) demonstrated that soluble antigens obtained by disintegration of Brucellae by sonic vibration or by a disintegrator reacted in agar gels with antisera and produced with them precipitation bands. On the other hand, Sulitzeanu(4) showed that nonsoluble particles resulting after disintegration of the majority of cells and after removing the rest of the intact bacteria by centrifugation, although unable to produce precipitation bands in agar gels, absorb agglutinins, while the soluble antigens do not possess this ability. Since in these experiments only *Brucella suis* was employed, the following experiments were carried out with *Br. suis, Br. abortus* and *Br. melitensis* to ascertain whether the antigens involved in these 2 different seroreactions are specific or not.

*Methods.* The antigens were prepared by the method of Olitzki and Sulitzeanu(3). From the 2 methods of immunization described by them the subcutaneous method with employment of an adjuvant was preferred. The bacteria were repeatedly disintegrated in a disintegrator (Mickle, Mill Works, Gomshall, Surrey) and remaining intact bacteria and cell walls were removed by centrifugation. For the agglutination test with intact bacteria acetone dried bacteria suspended in physiological saline were employed. 0.04 mg/ml of dried bacterial substance still gave a measurable opacity (O.D. 0.014) at 660 mμ and a visible agglutination. Cell wall antigens were prepared from disintegrated bacteria by centrifugation. At first the intact bacteria were removed by repeated centrifugation at 3400 rpm until no more sediment was produced. Then the supernatant was centrifuged 30 minutes at 9000 rpm. The absence of intact bacteria was verified by microscopical examination. The resuspended sediments gave opalescent suspensions, and

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TABLE I. Absorption of Precipitins from 1 ml of *Brucella melitensis* Antiserum Diluted 1:4.

| Absorbing antigen prepared from | Absorbing quantity (mg/ml) | No. of bands appearing in agar gel with *Br. abortus* | *Br. suis* | *Br. melitensis* |
|---|---|---|---|---|
| *Br. melitensis* | 2.4 | 0 | 0 | 0 |
| | 1.2 | 0 | 0 | 1 |
| | .6 | 0 | 0 | 1 |
| | .3 | 0 | 1 | 2 |
| | .15 | 0 | 1 | 2 |
| *Br. suis* | 2.4 | 0 | 0 | 0 |
| | 1.2 | 0 | 0 | 1 |
| | .6 | 0 | 0 | 1 |
| | .3 | 0 | 1 | 2 |
| | .15 | 1 | 1 | 2 |
| *Br. abortus* | 2.4 | 0 | 0 | 0 |
| | 1.2 | 0 | 0 | 1 |
| | .6 | 0 | 0 | 1 |
| | .3 | 0 | 1 | 3 |
| | .15 | 1 | 1 | 3 |
| Control, not absorbed | | 3 | 3 | 3 |

for agglutination tests higher optical densities of at least 0.074 were required. The best results were obtained at O.D. 0.124. The cell wall agglutination was different in its appearance from the agglutination of intact cells. Large flakes appeared and sedimented slowly in contrast to the granular, small flaking type of bacterial agglutination.

*Results.* Table I shows results of an absorption experiment with *Br. melitensis* and *Br. abortus* immune sera. By absorption with intact bacteria almost the same results were obtained as those obtained by absorption with cell walls. After addition of 25 mg of intact dried bacteria or dried cell walls to 5 ml of immune serum diluted 1:10 almost all agglutinins were removed when the homologous antigen was employed. However, when the heterologous antigen was employed, *e.g., Br. melitensis* in *Br. abortus* antiserum, then only the antibodies for the heterologous antigen were removed, and there still remained antibodies for the homologous antigen. Thus cell walls exhibit the antigenic scheme described by Miles and Wilson(5) for intact bacteria, according to which there is a prevalence of antigen M over antigen A in *Br. melitensis* and a prevalence of A over M in *Br. abortus.*

The result of absorption with moderate quantities of antigen must, therefore, be the production of a monospecific antiserum anti M for *Br. melitensis* and anti A for *Br. abortus.*

Completely different results were obtained in absorption experiments with bacterial extracts liberated from insoluble cellular components by centrifugation. These antigens were completely non-specific, since by employment of any of the 3 antigens in sufficient quantities all antibodies could be completely removed.

Table II shows that after absorption of *Br. melitensis* antiserum diluted 1:4 with minimal amounts of disintegrated bacterial substance as 0.6 mg/ml the majority of precipitating antibodies have already disappeared. When 2.4 mg/ml of the absorbing antigen were employed, this process was complete and no more precipitation bands appeared. The quantitative differences between these 2 reactions are evident. For incomplete absorption of heterologous agglutinins from 5 ml of se-

TABLE II. Absorption of Agglutinins by Cell Walls and Intact Bacteria.

| Immune serum | Absorbing antigens | | Reciprocals of agglutination titers with Intact bacteria *Br. melitensis* | Intact bacteria *Br. abortus* | Cell walls *Br. melitensis* | Cell walls *Br. abortus* |
|---|---|---|---|---|---|---|
| *Br. melitensis* | *Br. melitensis* | i.b. | 20 | 10 | 20 | 0 |
| | *abortus* | " | 200 | 20 | 200 | 20 |
| | *melitensis* | c.w. | 20 | 20 | 20 | 0 |
| | *abortus* | " | 200 | 20 | 200 | 20 |
| | Not absorbed | | 2,000 | 2,000 | 1,000 | 2,000 |
| *Br. abortus* | *Br. melitensis* | i.b. | 20 | 1,000 | 20 | 500 |
| | *abortus* | " | 10 | 0 | 20 | 20 |
| | *melitensis* | c.w. | 20 | 1,000 | 20 | 500 |
| | *abortus* | " | 0 | 50 | 20 | 20 |
| | Not absorbed | | 1,000 | 2,000 | 500 | 1,000 |

i.b. = intact bacteria; c.w. = cell walls. 0 = agglutination negative at dilution 1:10.

rum diluted 1:10, 25 mg of total bacteria or cell walls were required, which corresponds to 50 mg of absorbing substance for 1 ml of undiluted serum. For complete absorption of all precipitins from 1 ml of a serum diluted 1:4 2.4 ml of disintegrated bacterial substance were required, which corresponds to 9.6 mg / 1 ml of undiluted serum.

*Summary.* Bacterial agglutinins were removed partly by intact bacteria or cell walls from brucella antisera and by absorption with heterologous antigens monospecific antisera were produced. Cell wall agglutination parallels almost completely the agglutination of intact bacteria, when cell wall suspensions of high density were employed. Precipitins for soluble bacterial antigens demonstrable by agar gel precipitation technic were nonspecific and were removed by relatively small amounts of absorbing antigens.

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## Indirect Effect of X-Irradiation on $I^{131}$ Uptake in Chick Embryos.* (24953)

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Changes found in metabolism of $I^{131}$ by thyroid in various animal species following total or partial body exposures to ionizing radiations are attributed to possible systemic response to exposures(1-6), but the evidence offered in support of this idea is circumstantial in nature. The following data afford direct evidence that changes in $I^{131}$ uptake by thyroid in chick embryos are indirectly mediated by a substance found in peripheral circulation shortly after exposure of embryos to ionizing radiations.

*Materials and methods.* Fertilized eggs of White Plymouth Rock strain were obtained from commercial supplier. All injections were made into the air sac. Exposures to 600 r of x-rays were made approximately one hour after injections. Factors were 250 kv, 15 ma, Al parabolic plus 0.5 mm Cu with a HVL of 2.15 mm Cu, 25 cm Target-skin distance, and output 20.3 r/min. Approximately 24 hours after exposures and/or injections, the neck sections containing the thyroid were dissected out and counted for one minute in scintillation type well counter. The following experimental procedures were done. In Exp. 1, 12 or 15-day-old eggs were injected with 5 $\mu$c or 2.5 $\mu$c of $I^{131}$, respectively, about one hour before exposure to 600 r of x-rays. Controls received $I^{131}$ only. In Exp. 2, 5 $\mu$c of $I^{131}$ and 0.1 ml of pooled sera obtained from 13½ and 14-day-old embryos that had been exposed to 600 r of x-rays on 13th day were injected into 14-day-old eggs. Controls were injected with 5 $\mu$c of $I^{131}$ and 0.1 ml of normal saline. In Exp. 3, 2.5 $\mu$c of $I^{131}$ and 0.1 ml of pooled sera obtained from 15½-day-old embryos that had been exposed to 600 r of x-rays at 15 days of age were injected into 15-day-old eggs. Controls were injected with 2.5 $\mu$c of $I^{181}$ and 0.1 ml of pooled sera obtained from untreated 15½-day-old embryos.

*Results.* The data shown in Table I indicate that not only does exposure of embryos to 600 r of x-rays result in increased uptake of $I^{131}$ by the thyroid, but that pooled sera obtained from embryos exposed to 600 r of x-rays and injected into untreated eggs results

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