Summary. A column of pulverized Mealorub rubber has been adapted successfully to the quantitatively accurate chromatographic separation of a mixture of crystalline estrone, estradiol and estriol. When 20 ml each of 20, 40 and 60% aqueous methanol (v/v) are passed successively through the column, estriol is eluted by the 20%; estradiol by the 40%, and estrone by the 60% concentration of methanol.

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A Non-specific Brucella Agglutinating Substance in Bovine Serum.*† (18817)

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In evaluating the results of the serum agglutination test for brucellosis in cattle, animals with titers ranging from incomplete agglutination at the 1:50 dilution to incomplete at 1:100 are designated as suspects, and herds containing one or more such animals, with all other animals negative to the test, are considered as suspicious or suspect herds. Animals with higher titers are considered infected and are designated as reactors. In a brucellosis control plan in Minnesota which is based on county areas and requires periodic testing of all the cattle in the controlled counties, a ratio of approximately two suspect herds to three reactor herds is usually disclosed. In counties where the percent of infection is very low, the number of suspect herds may exceed appreciably the number of reactor herds. Moreover, the majority of suspicious herds are so classified because of the presence of a single suspect animal. These observations coupled with the fact that approximately 85% of the animals displaying suspicious titers in suspect herds are found to be negative to subsequent retests have suggested that non-specific agglutinins may be responsible for a large portion of these reactions.

In the course of trying to demonstrate nonspecific agglutinins for Brucella a filter paper chromatographic technic was devised that made possible the detection of an agglutinating substance capable of reacting with Brucella test antigen but differing markedly from specific or high-titered agglutinins in its affinity for adsorption on cellulose. The test was based on two recently published observations; first, Franklin and Quastel(1) showed that certain proteins together in solution could be separated by filter paper chromatography when the proper developing solution and a means of localization of the fractions were employed; and second, Castaneda(2) observed that Brucella agglutinating serums were capable of fixing a stained Brucella antigen to the surface of filter paper.

Materials and methods. Filter paper. The paper used in this test was S & S 589, White Ribbon. It was cut in strips 3½ inches long and ¾ of an inch in width. *Developing solution.* The solution found to give the best results was potassium acid phthalate (0.05 M) adjusted to pH 6.2 with sodium hydroxide. *Antigen.* A hematoxylin-stained Brucella antigen prepared according to the method of Roepke(3) for use in the milk and cream ring test was used exclusively in the test. The antigen was found to give better results if the

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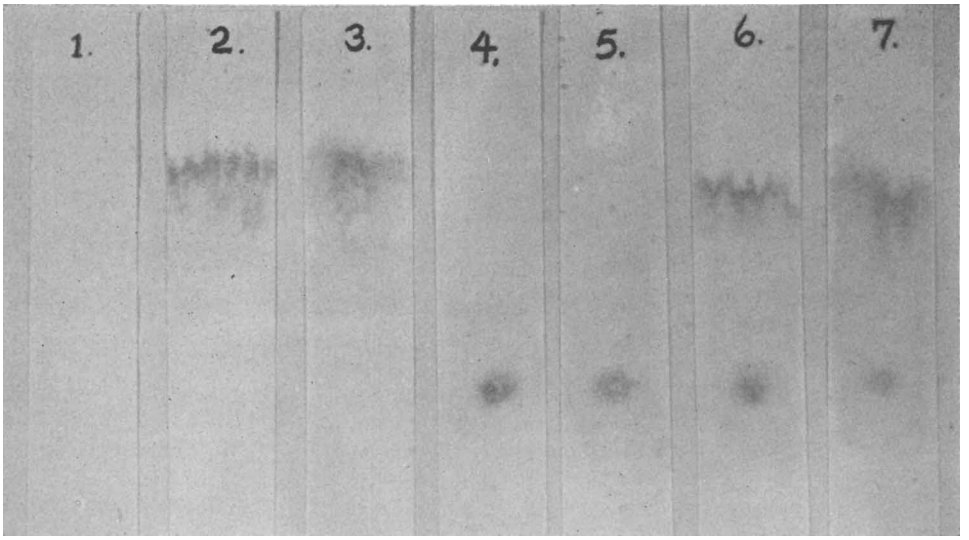

FIG. 1. Chromatographic tests for specific and non-specific Brucella agglutinating materials in bovine serum. No. 1 negative, No. 2 and 3 specific reactions, No. 4 and 5 non-specific reactions and No. 6 and 7 mixed reactions.

glycerin was removed by washing with saline in the centrifuge and resuspending to the original concentration in 0.5% phenolized physiological saline.

The test was performed in the following manner: 1. The paper strip was placed on a smooth, nonabsorbent surface. Using a 0.2 ml serological pipette held in a vertical position with its tip pressed firmly against the paper, 0.02 ml of serum was placed about an inch from one end of the strip and allowed to soak slowly into the paper. This resulted in the formation of a spot about 18 mm in diameter. The strip was hung with the tip, just below the serum spot immersed in the developing solution. It was then allowed to develop by capillary ascent. 2. After 10 to 15 minutes, the strip was removed from the solution, and by means of a small camel's hair brush the antigen was applied to the entire path of migration of the serum. It was necessary to prevent the antigen from contacting the dry area of the paper, for from there it could not readily be washed. 3. The strip was placed immediately on a towel or other absorbent surface and washed. The washing was accomplished with 1% saline applied with a second brush in a dabbing or blotting motion. Areas of the strip where antigen and antibody had combined could withstand vigorous and prolonged washing without removing all of the stained antigen.

Fig. 1 illustrates the types of reactions that were encountered. Strip 1 shows the reaction obtained with negative serums. All of the antigen was readily washed from the paper. On strips 2 and 3 the agglutinins advanced up the paper with the solvent as was evidenced by the fixing of the antigen near the top of the strips. This type of reaction was designated as specific, for it was the type given by high-titered bovine serum as well as the majority of low-titered serums from infected herds and also by serums of animals treated with Strain 19 Brucella vaccine. Serums from humans suffering from brucellosis as diagnosed clinically and confirmed by agglutination and blood culture also give this type of reaction. On strips 4 and 5 the agglutinating material remained at the spot where the serum was applied. The reaction was designated as non-specific because the agglutinating substance involved was found to adsorb on a variety of cellulose materials, and in this respect differed from the specific or gamma globulin antibody. Quite a large number of serums were found to display both kinds of agglutinins. Strips 5 and 6 serve as examples.

TABLE I. Summary of Differential Tests on Bovine Serums.

Source of serum	No. of animals tested	Filter paper reaction		
		No. specific	No. nonspec. only	% non-spec. only
Suspects from reactor herds	197	188	9	4.6
Suspects from suspect herds	220	79	141	64
Negatives from negative herds	100	0	12	12

A similar reaction was obtained by mixing serums of the specific and non-specific types.

Experimental. The following bovine serums were tested by the filter paper chromatographic method: 197 suspect serums from animals in herds which also contained reactors, 220 suspect serums from animals in otherwise negative herds, and 100 samples that were adjudged negative on the basis of the routine plate agglutination test in which 1:25 was the lowest dilution tested.

Results. Because a significant percentage of serums showing the specific reaction on filter paper also contained some non-specific agglutinating material, the non-specific was considered to be of importance only when it was the sole cause of the fixation of antigen on the paper. The results recorded in Table I adhere to that criterion.

It may be observed that 64% of the suspect serums from animals in otherwise negative herds gave the non-specific reaction only, while the same was true for less than 5% of the suspect serums from herds containing reactors.

Because 12 of the so-called negative serums gave non-specific reactions on paper, a portion of that group was subjected to a tube agglutination test beginning at a dilution of 1:4. The results indicated the sensitivity of the filter paper test to be equivalent to approximately the 1:16 dilution of the tube test.

The reliability of the test is being checked by following the serum agglutination retests on suspect animals from otherwise negative herds. This phase of the study is still in progress. However, the results obtained thus far are worth reporting. Of 53 suspects that gave non-specific reactions on the original herd test, 50 were declared negative to the serum agglutination retest. Two retained a suspicious titer, and 1 was reported as increasing in titer to the reactor level. This increase in titer was from incomplete agglutination at 1:100 to complete at 1:100. In contrast to the above results, out of 34 suspect animals in otherwise negative herds that originally gave specific reactions on filter paper, 16 have become negative, 11 have remained suspicious and 7 have become reactors.

Discussion. It is believed that the non-specific agglutinating substance is present in varying amounts in a large portion of animals of a variety of species. Its presence has been detected, as reported here, in a large number of bovine serums. Work still in progress indicates that more than 90% of normal swine contain the substance in detectable amounts in their serums. A similar substance also appears to be present in human serums. These latter findings are being expanded.

Attempts are being made to obtain the non-specific agglutinating material in an amount and state of purity that will enable further characterization of the substance. Adsorption on cellulose or Brucella organisms followed by elution with distilled water has yielded preparations showing a marked increase in concentration as indicated by agglutination titer. In a second line of approach, the methods for the fractionation of plasma presented by Cohn *et al.*(4) have been used. The non-specific substance is not extracted with the gamma globulin fraction as is the case with specific Brucella agglutinins. Instead, the substance with its agglutinating activity still intact is found in the fraction which Cohn has designated as containing isoagglutinins, prothrombin, and cold insoluble globulins.

The fact that readily demonstrable amounts of the non-specific agglutinating substance are present in many serums of apparently normal animals indicates that it may be a normal

4. Cohn, E. J., Gurd, F. R. N., Surgenor, D. M., Barnes, B. A., Brown, R. K., Derouaux, G., Gillespie, J. M., Kahut, F. W., Leever, W. F., Lui, C. H., Mittelman, D., Mouton, R. F., Schmed, K., and Uroma. E., *J. Am. Chem. Soc.*, 1950, v72, 465.

serum constituent. In a low percentage of animals, sufficient amounts are present to cause interference with the application of the standard agglutination test at the 1:50 and 1:100 dilutions.

Summary. A filter paper chromatographic technic was used to demonstrate the presence in animal serums of a non-specific agglutinative material for Brucella. As judged by the physio-chemical properties of the material it does not seem to be related to agglutinins which arise from a specific antigenic stimulus of the classical type.

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Augmentative Effects of Steroids and Chorionic Hormone on Rat Ovary. (18818)

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It long has been known that the preliminary administration of estrogenic substances results in a marked augmentation of ovarian weight induced in rats by a gonadotrophic hormone. This was first accomplished in hypophysectomized rats(1-3), and in immature females not only with estrogens(4) but with testosterone propionate(5). During the war years Dutch students(6) also approached this problem and from their results drew an elaborate theory regarding gonadal function. The purpose of the present report is to direct attention to these studies and to add further findings with various steroid substances.

The rats employed in this study were from the Stanford colony and weighed from 35 to 40 g at 21 to 24 days of age. The usual procedure was to administer a single subcutaneous injection of the steroid or control material on the first day of the experiment while on the second day they were given 3 intraperitoneal injections of 250 I.U. of chorionic hormone (A.P.L.--Ayerst). The 2 sites of injection were used in order to avoid any change in the rate of absorption of the active material. The rats were sacrificed with natural gas on day 6 when body, uterine and ovarian weights were obtained, and the ovaries fixed in Bouin's solution for section and microscopic examination.

TABLE I. Chorionic Hormone 750 I. U. Given on Day 2 to Each Group.

Day 1	No. Animals	Avg body wt, g	Uterus, g	Ovaries, g
Sesame oil .1 cc	4	42	.140	.023
Cottonseed oil .1 cc	5	40	.113	.024
Sesame oil .5 cc	4	46	.105	.037
Pregnenolone 5 mg	4	44	.131	.032
Cholesterol 25 "	4	43	.150	.042
Stilbestrol .5 "	5	39	.075	.059
" 1 "	4	44	.083	.071
Estrone 5000 I. U.	4	46	.119	.039
" 10000 I. U.	4	49	.081	.059
Estradiol benzoate .8 mg	4	44	.095	.072
" dipropionate 2 mg	4	44	.114	.060
Progesterone 5 mg	5	38	.145	.018

The injection of sesame or cottonseed oil 0.1 cc on the first day of the experiment gave ova-

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