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Purified Protein Antigen from Brucella.

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Several preparations have been employed in determining allergy to the brucella group.[1-5] We have tried to obtain a standard preparation which could be measured accurately, would be a good antigen and which could be easily prepared with the least possible denaturation. This has been made possible by following a modification of Seibert's method[6] for the preparation of P.P.D. (purified protein derivative from tuberculin).

Since the brucella organisms cannot be made to grow on synthetic media, as previously demonstrated by investigators,[7] we were unable to utilize a filtrate of the cultures as with the tuberculin products.[6] We decided to start with a solution of the cellular constituents of the brucella organisms and proceed to extract and purify the protein fraction. Care was taken in selecting our methods and materials to make them as simple and economical as possible. A known strain of *Brucella abortus* (strain No. 456, National Institute of Health, Washington, D. C.) was selected for our experiment.

Large amounts of *Br. abortus* are grown on standard nutrient agar pH 7[8] in Blake bottles for 72 hours at 37°C. Approximately 5 ml of saline with 0.5% phenol are added to each bottle and left for 30 minutes in contact with the culture to loosen the cells. With sterile pipettes the phenolized saline containing the suspension of cells is removed from the bottles and the surface of the agar

1 McFadyean, J., and Stockman, S., Report of the Departmental Comm. appointed by Bd. of Agriculture and Fisheries. Appendix to Part I, London, His Majesty's Stationery Office, 1909.

2 Burnet, Et., *Arch. de l'Inst. Pasteur de Tunis*, 1922, **2**, 165.

3 Giordano, A., *J. Am. Med. Assn.*, 1929, **93**, 1957.

4 Hershey, A. D., The chemistry of Brucella. Thesis. Mich. State College, unpublished (After Huddleson's "Brucella infections in animals and man"), 1933.

5 Huddleson, I. F., Johnson, H. W., and Hamann, E. E., *J. Am. Vet. Med. Assn.*, 1932, **36**, 16.

6 Seibert, F. B., *Am. Rev. Tuberc.*, 1934, **30**.

7 Huston, R. C., Huddleson, I. F., and Hershey, A. D., Tech. Bull. No. 136, May, 1934, Michigan State College.

8 Wadsworth, A. B., *Standard Methods*, page 90, 1927 edition.

thoroughly washed with more phenolized saline. The organisms are recovered by centrifuging at high speed; then they are crushed with sand in an electrical grinding apparatus for about 2 hours or more. The mixture of sand and broken cells is mixed with 2 liters of 0.02 N sodium carbonate and placed in the refrigerator overnight. Next morning the alkaline suspension is filtered, first through paper and then through a Berkefeld candle. To the filtrate we add 0.5% phenol to avoid bacterial contamination during the following steps of the procedure. The filtrate is then concentrated by ultrafiltration through alundum cups impregnated with 13% gun-cotton-glacial acetic acid solution prepared according to the method described by Seibert.[6] The ultrafiltration is continued until the liquid is concentrated to about 100 ml; then it is washed by passing about 500 ml of distilled water through the alundum cup filter. The concentrated colloidal solution is then filtered through a Berkefeld candle to eliminate any insoluble material that may have sedimented during the ultrafiltration. The protein is precipitated by mixing the colloidal solution with one-fourth its volume of a 50% freshly prepared aqueous solution of trichloracetic acid, making a final concentration of 10% acid. After standing overnight in the icebox the protein precipitate is thrown down by centrifugation. Then it is washed repeatedly by centrifugation with freshly prepared 10% trichloracetic acid solution, until the washings are colorless. The protein product is then washed with large volumes of anhydrous ether by repeated triturations and centrifugations until all the acid is removed and the precipitate is completely dehydrated.

The final protein product thus obtained from *Br. abortus* is a fine light brown powder, which is not completely soluble in water, but is easily dissolved by adding a few drops of 0.1 N alkali. The solution can be neutralized with N/10 hydrochloric and it remains clear. The uniform chemical composition of the product obtained is shown in Table I.

TABLE I.

	Nitrogen % dry weight	Carbohydrate (unhydrolyzed) % dry weight
1	12.35	1.02
2	12.74	1.47
3	14.35	1.19

The chemical composition of the antigen is fairly constant, the nitrogen and polysaccharide contents of protein prepared from different batches of cell cultures varying within approximately 1 or 2%.

The antigen prepared by this method gives positive biuret and Millon's tests and a positive xanthoproteic reaction. It is not coagulated by heat. Intracutaneous test in guinea pigs which have been previously infected with different strains of brucella organisms showed that a definite skin reaction lasting more than 48 hours can be produced when 0.005 mg of the purified protein is injected. Larger doses may produce necrosis or even death of the animals, depending on the degree of sensitization. Sera of rabbits inoculated with *Brucella* give a positive precipitin test and a positive complement fixation test when purified brucella protein is used.

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Alteration of Chromosome Sensitivity to X-rays with NH_4OH.

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Previous experiments have led the writer to attribute the formation of chromosome abnormalities by X-rays to the action of electrons on the positively charged materials of the chromosomes.[1, 2] According to this hypothesis, it is to be expected that if the sign of the electrical charges carried by the X-ray-sensitive materials of the chromosomes could be changed, the sensitivity of chromosomes to alterations by X-rays would be very much reduced. If the pH of the medium surrounding the sensitive substance could be shifted to the alkaline side of its isoelectric point or to the alkaline side of the pK of those of its constituent groups responsible for the X-ray reaction, the ionization of these positively charged groups would be suppressed and only the negatively charged groups would be left ionized. Thus it is to be expected that penetrating bases, if they are strong enough to change the intracellular pH sufficiently, should reduce the sensitivity of the chromosomes to X-rays. Penetrating acids, on the other hand, should have no effect unless the pH was shifted to such an extent that new chromosome materials or their constituents were thus brought to the acid side of their isoelectric points and therefore rendered electropositive. Substances behaving in this manner would most likely be proteins. Furthermore, if the pH of the intracellular medium surrounding the chromosomal ma-

[1] Marshak, A., *Proc. Nat. Acad. Sci.*, 1937, **23**, 362.
[2] Marshak, A., and Hudson, J. C., *Radiology*, 1937, **29**, 669.