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17200. A Readily Soluble form of P. B. P. for Use as a Routine Diagnostic Test.

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We reported the preparation of a purified protein antigen from *Brucella,*[1] made by following a modification of Seibert's method for the preparation of PPD (purified protein derivative from tuberculin). This protein substance was a fine, light brown powder of fairly constant chemical composition, not completely soluble in water but easily dissolved by the addition of a few drops of 0.1 N alkali. The solution could be neutralized with 0.1 N HCl and remained clear. This preparation was used by us, as well as by other investigators, for the study of cutaneous hypersensitiveness to *Brucella.* As workers may not have laboratory facilities, we have devised a method whereby the PBP (purified brucella protein) is put up in vial form and in just the right amount for skin testing. By adding a measured quantity of sterile saline buffered solution, the PBP is ready for use.

To prepare the PBP in this way, a weighed amount of the protein antigen is dissolved, as indicated above, to make a concentration of one mg per cc. This solution is dialized through cellulose tubing (Visking Corp.) in cold water for 24 hours. Any impurity that may separate is removed by centrifugation. Then 50 mg of Beta lactose per cc of the PBP solution are dissolved. The resulting liquid should be completely clear. One half cc of this solution, containing 0.5 mg of the *Brucella* protein extract, are bottled in glass vials (about 8 ml capacity) of the type ordinarily used for vaccines, and the contents dried by lyophilization. The lactose has no deleterious effect on the PBP; does not influence the allergic reaction, and is used only to give bulk to the product after drying, since the amount deposited in each vial is infinitesimal.

For skin testing, 5 ml of the sterile diluting fluid are added to each vial, by means of a sterile syringe and needle through rubber stoppers of the vial, and shaken for 2 or 3 minutes to dissolve their contents. The diluting fluid is the same used for Seibert's PPD[2] and is a buffered saline liquid made from the following solutions:

KH_2PO_4: 9.078 g dissolved in 1000 cc of distilled water

$Na_2HPO_4 \cdot 2H_2O$: 11.876 g dissolved in 1000 cc of distilled water

Two parts of the solution of the potassium salt are mixed with 8 of the sodium salt, and 0.25% phenol is added.

Each vial contains enough PBP for 50 tests. The tests are performed by injecting 0.1 ml of the diluted protein product with a tuberculin type syringe and a 26-gauge needle into the skin over the flexor surface of the forearm. The site of the injection is examined 48 hours later. Subjects who react positively show a pronounced erythema and edematous induration. Negative cases show no changes.

This preparation was tested on 25 individuals supposed to have been in contact with infected material. Two of them were veterinarians and 2, their assistants. The rest were milkers and dairy workers. Of this group 8 gave positive and 17, negative.

[1] Morales-Otero, P., and González, Luis M., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 703.

[2] Seibert, F. B., *et al., Am. Rev. Tuberculosis,* 1934, **30**, 707.

As a further check of the specificity of this lyophilized form of PBP, 10 patients from the University Hospital, who had been diagnosed as brucellosis cases and who had previously given a positive skin test with the old form of PBP, were again tested with this readily soluble form. All gave a positive skin reaction.

For negative control group, 20 healthy subjects, selected at random from the general population and with no history of brucellosis or symptoms usually associated with this condition, were also tested; all were negative to the lyophilized PBP.

Summary. A purified brucella protein, subjected to further purification and presented in a soluble lyophilized form, has been described. Weighed amounts are placed in glass vials, which, after the addition of a measured quantity of sterile saline buffered solution, produces a product ready for use. This procedure facilitates the use of the product in epidemiological investigation and as a routine diagnostic test.

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17201. Effects of Bone Extracts Injected into the Mouse Testis.*

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Bone will develop from bone marrow when it is transplanted under certain conditions. This subject has recently been reviewed by Levander[1] and Pfeiffer.[2] However, these two authors differ markedly in their interpretation of how bone is formed from the marrow transplant. Levander[1] has reported that when bone marrow is transplanted, all of the grafted cells disintegrate and mesenchymal cells from the surrounding tissue soon migrate into the graft and are transformed into chondroblasts and osteoblasts which form cartilage and bone. Pfeiffer[2] agrees with Levander that the hematogenic and myelogenic cells disappear from the graft but believes that when bone forms, the marrow reticulum cells of the graft survive and these cells develop into osteoblasts just as they do in the marrow cavity when medullary bone forms there as described by Bloom, Bloom and McLean[3] in the pigeon.

The evidence in the literature[4-7] that there is an extractable osteogenic substance would tend to support Levander's hypothesis. However, it has been questioned whether such a substance exists, and evidence that injury is the primary factor in causing bone to form when bone extracts are injected has been advanced by Heinen.[8] Apparently mesenchymal cells transform into osteoblasts in either case. It therefore becomes important to test whether the mesenchymal cells present in the area where bone developed in marrow grafts would produce bone under the same or greater injury than resulted from grafting, plus the extract from a similar or larger piece of marrow than was grafted.

Materials and methods. Mice of the Strong[9] A strain were used in this experiment because it has been shown that bone will develop in marrow grafts in the anterior chamber of the eye and in the testes of mice of

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1 Levander, G., *Acta Chir. Scand.*, 1940, **83**, 545.

2 Pfeiffer, C. A., *Anat. Rec.*, 1948, **102**, 225.

3 Bloom, W., Bloom, M. A., and McLean, F. C., *Anat. Rec.*, 1941, **81**, 443.

4 Annersten, S., *Acta Chir. Scandinav.* (Suppl. 60), 1940, **84**, 1.

5 Annersten, S., *Chirurg.*, 1941, **13**, 76.

6 Bertelsen, A., *Acta Orthopaedica Scandinavica*, 1944, **15**, 139.

7 Levander, G., and Willistaedt, H., *Nature*, 1946, **157**, 587.

8 Heinen, J. H., personal communication, 1948.

9 Strong, L. C., *J. Hered.*, 1936, **27**, 21.