made in each. The weight of the elements was not taken since the author undertook this work from the standpoint of histological study of their effect on the tissues. The approximate size of the pieces of sodium, lithium, potassium, phosphorus and thallium placed at each site of implantation was 5 x 5 x 2 mm. and from one to 2 times this amount was used in the case of the other elements, with the exception of bromine, in which instance one cubic centimeter was placed in each area of implantation. The most striking feature of the work was the fact that a large number of the elements were practically inert when placed in the tissues. In this work the author hopes to correlate some of the physical properties of the elements with the tissue reactions and their histological study after a more lengthy series has been run. The reason why a lengthy series is necessary is because of the difficulty of sterilization in the case of some of the elements and also of keeping the animal wound sterile.

5599

Papain Preparations Suitable for the Prevention of Adhesions.

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The use of proteolytic enzymes in the surgical treatment of adhesions has indicated a definite superiority for the vegetable enzyme, papain.[1, 2, 3, 4] This report is concerned with the development of a sterile papain preparation suitable for clinical use, the standardization of the product and some descriptive aspects of its behavior in the peritoneum.

The two procedures previously described for the preparation of a sterile trypsin product[5] have been applied to papain with varying success. The simplest method, namely, the pressure filtration of a glycerine extract has been discarded as unsuitable for clinical purposes. Even though filtered glycerine extracts of trypsin lose very

[1] Kubota, *Mitteilungen aus der Medizinischen Facultat der Kaiserlichen Kyushu-Universtat*, 1924, Bd.9, Heft 2.

[2] Ochsner and Mason, PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 524.

[3] Ochsner and Herrmann, *Arch. Surg.*, 1922, **17**, 365.

[4] Ochsner and Garside, yet unpublished.

[5] Walton, *J. Pharm. and Exp. Ther.*, 1930, **40**, 403.

little activity in the course of 8 months' storage and glycerine extracts of papain show a high degree of heat stability, the storage behavior of the latter is unfavorable. About 50 assays on 12 different lots over periods up to 4 months showed that filtrates stored open in the refrigerator averaged a 50% activity-loss in 1 month and ampouled filtrates stored in the refrigerator averaged a 50% activity-loss in 4 months. The cause for this deterioration was not determined although a number of possibilities were examined such as atmospheric oxidation, contact with mercury vapor, exposure to ultra-violet light and absorption of alkali from the container.

The second method, previously described, consisted in filtration of aqueous or glycerine extracts through Berkefeld filters (N) and precipitation of the active product with alcohol and ether. This presented no unexpected difficulty when applied to papain and the powdered products have shown consistent stability on storage. The firm of Parke Davis & Co. have cooperated in producing sterile and standard preparations by this method. The activity of the standardized, sterile product corresponds closely to that of the ordinary stock product, mgm. for mgm.

Papain preparations intended for oral administration have been assayed in drug houses by a method involving the digestion of raw meat. Further definitions of activity were considered desirable when standardizing preparations for this purpose. A method has been used based on formol titrations before and after digestion of purified gelatine. Another method, not as precise but simpler, is based on the time for disintegration of glycerine-stored fibrin. A third method used is a modification of the procedure described by Gates[6] and Gilman and Cowgill,[7] based on the extent of digestion of the gelatine on a strip of photographic film. In each of these methods citrate solutions have been used as "activator."

Because the effectiveness of papain was exhibited in such remarkably low activity-concentrations, Kubota[1] ascribed the effect to a chemotaxis with respect to leucocytes, leucoproteases presumably digesting the exudative fibrin. From our studies demonstrating the high degree of stability of papain it is suggested that the proteolytic activity of the papain itself might be adequate to explain its behavior. Aqueous solutions at 1-1,000 concentration were shown to retain about 55% of the original activity after standing 40 hours at 40°. This degree of stability was exhibited both by the purified

[6] Gates, *Science*, 1930, **72**, 147.

[7] Gilman and Cowgill, *J. Biol. Chem.*, 1930, **88**, 743.

sterile product and the ordinary stock product. Trypsin solutions (Fairchild's standard preparation), in contrast, lost 95% activity in 3 hours under the same conditions. Papain solutions were unaffected by the presence of serum in sufficient concentrations to inhibit trypsin almost completely at the same activity concentration. In the peritoneal cavity of dogs, papain solutions at concentrations of about 1-15,000 retained 50% activity after 5 hours.* Under similar conditions, the activity of trypsin disappears completely within 2 hours.[5]

The minimal fatal concentration for normal dogs may be placed at 1-2,000, when using 1 liter of isotonic saline per 10 kg. dog. At concentrations up to 1-300 there is marked irritation of the peritoneal surfaces and some hemorrhage. However, the amount of blood thus liberated, as indicated by hemoglobin determinations, has never exceeded 12 cc. and is thus not sufficient to explain the cause of death. Other possible explanations are being studied. Concentrations of 1-15,000 cause some irritation and occasionally slight bleeding, the amount of blood rarely exceeding 1 or 2 cc. These conclusions are based on experiments with 16 dogs. The extent of irritation caused by papain and by trypsin is about the same mgm. for mgm., as determined in 12 rats at concentrations of 1-2,000 and of 1-10,000. In this higher dilution there is little or no irritation with either. By way of reference it may be pointed out that papain has been considered effective in concentrations of 1-400,000.[1, 2, 4]

5600

Effect of Carbon Dioxide on Ethylene and Nitrous Oxide Anesthesia.

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As a continuation of the investigation of the effects of CO_2 in anesthesia, this report embodies the results of a study of the effect of this gas on anesthesia by ethylene and by nitrous oxide. As shown in the accompanying graphs, it has been found that CO_2 exerts an effect on anesthesia by these gases of the same nature

* Determined by activating removed samples with H_2S and assaying by the photographic film method. Addition of H_2S increases the activity about 36 times.