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Mouse Pathogenicity with Gastric Mucin as an Index of Staphylococcus Virulence. (22657)

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Bacterial virulence, defined broadly as the capacity of an organism to invade and multiply in tissues and produce disease or death, is best measured by observing pathological effects induced by the microorganism in a suitable host. Animal virulence tests, although employed for a number of other bacterial species, have not been applied to staphylococci chiefly because of their inconstant and irregular pathogenicity for laboratory animals. Such biochemical properties as coagulase, pigment and hemolysin production as well as mannite fermentation are utilized instead with the greatest reliance being placed on the first, but the validity of these criteria as indices of virulence in all instances has been subject to serious question by many investigators. Lack and Wailling(1), as one example, in a study on the factors contributing to pathogenicity of staphylococci, state, "While there is strong evidence that the absence of coagulase production is associated with nonpathogenicity, there is no evidence that all coagulase-producing strains are pathogenic."

Hog gastric mucin has found wide application as an adjuvant in converting infections caused by organisms considered of low virulence for mice on intraperitoneal injection into rapidly lethal ones. Among the numerous bacterial species reported amenable to its action in this regard are some strains of staphylococci(2-4). Ercoli *et al.*(5) have shown that staphylococci injected intraperitoneally into mice with mucin remain viable and spread from the abdominal cavity, while without it they are rapidly destroyed. It was believed of interest to ascertain whether the mouse could be converted from a relatively resistant host to staphylococcus infection by the intraperitoneal route into one readily susceptible, by means of the adjuvant, and if so, to determine whether this procedure could be adapted to the differentiation of virulent from avirulent members in the same manner that animal inoculations are employed with corynebacteria, clostridia, mycobacteria, etc. To facilitate this evaluation, the *in vitro* presumptive biochemical reactions of a series of strains of staphylococci were compared with their intraperitoneal pathogenicity for mice with mucin.

Materials and methods. 60 strains of staphylococci recently isolated from a variety of such clinical sources as suppurative lesions, blood, nose and throat, urine and skin were employed. 5% hog gastric mucin (Type 1701-Wilson) was prepared according to Miller(6). Ten albino Swiss mice, weighing approximately 15-20 g, were used for each bacterial strain, of which 5 received 0.5 ml of 5% hog gastric mucin and the remainder 0.5 ml of physiological saline intraperi-

toneally 15-20 minutes prior to the intraperitoneal injection into each mouse of 0.25 ml of a 16 hour veal infusion broth culture. Animals were observed 48 hours and all deaths were recorded. In addition, one group of 10 mice received 0.75 ml of mucin and another group of 10 mice received 0.75 ml of veal infusion broth intraperitoneally in order to test toxicity of mucin and broth alone without any fatalities being noted in either group.

Coagulase production was determined by tube test. Contents of an ampule of desiccated rabbit plasma (Difco) was suspended in 3 ml of distilled water and 2 drops of a 16 hour veal infusion broth culture of each strain under investigation was added to 0.5 ml of the plasma solution and incubated in a water bath at 37°C for 3 hours. A positive test was distinct clotting of the tube's contents. Presence of yellow to deep orange colonies after incubation at 37°C for 2 days and at room temperature near the light for 3 more days on a medium containing 2 parts 2% veal infusion agar and 1 part sterile homogenized milk indicated pigment production. Hemolysin production was manifest by distinct clear zones around bacterial colonies on 5% human blood agar plates within 72 hours at 37°C. Mannite fermentation was denoted by red colonies and the formation of a red color in the surrounding medium, after incubation at 37°C for 72 hours, on a veal infusion agar plate containing 1% mannite and Andrade's indicator.

Results. The significant enhancement in pathogenicity for mice achieved with the aid of gastric mucin with a considerable proportion of the bacterial strains studied is demonstrated in Table I. It may be noted from the table that whereas only 5 of the 60 strains were able to produce a mortality of at least 3 out of 5 mice without mucin, 42 proved lethal to the same degree with the adjuvant. Thus, by its use, the mouse was converted from a host resistant to staphylococcus infection via the intraperitoneal route into one readily susceptible with 37 strains. In no instance was any strain found pathogenic to mice without mucin and innocuous with it.

To facilitate comparison between *in vitro* and animal tests, all strains fatal to at least 3 out of 5 mice with mucin were arbitrarily classified as pathogenic and hence virulent. Accordingly, 42 of the 60 strains investigated were found virulent and 18 non-virulent. The number and proportion of microbial strains found mouse pathogenic or non-pathogenic in relation to their reactions to each of the presumptive *in vitro* tests is summarized in Table II. Examination of Table II reveals that although mouse pathogenicity with mucin was not in complete accord with the results of the biochemical tests, a degree of correlation ranging from high to fair did exist between both, depending upon the test. Excepting hemolysin production and varying with each criterion, strains reacting positively were generally pathogenic to mice, while the negative reactors were non-pathogenic in the main. As for hemolysin production, 73.8% of the 42 hemolytic strains proved pathogenic, as were 61.1% of the 18 non-hemolytic strains. The relationship between coagulase production and virulence as determined by the procedure reported upon is of particular interest for it provides experimental corroboration for the observation of Lack and Waillling(1), quoted above. Thus only 1 of the 11 strains that failed to produce coagulase proved pathogenic. On the other hand, though 41 of the 49 coagulase positive strains were pathogenic, the remaining 8 were found non-lethal to the experimental animals.

In light of the questionable validity of the biochemical indices in some instances and in view of a number of discrepancies noted between the *in vitro* reactions and mouse patho-

TABLE I. Enhancing Effect of Gastric Mucin upon Pathogenicity of 60 Strains of Staphylococci for Swiss Mice.

	Without mucin		With mucin	
Mouse mortality*	No. strains	% of total	No. strains	% of total
0/5	47	78.3	13	21.7
1/5	3	5.0	3	5.0
2/5	5	8.4	2	3.3
3/5	1	1.7	8	13.3
4/5	2	3.3	14	23.4
5/5	2	3.3	20	33.3

* Numerator = No. died. Denominator = Total No. animals inj.

TABLE II. Intraperitoneal Mouse Pathogenicity with Mucin of Staphylococci in Relation to Their *In Vitro* Reactions.

In vitro test	Reaction	Total strains	Mouse pathogenicity with mucin: Pathogenic No.	Pathogenic % total	Non-pathogenic No.	Non-pathogenic % total
Coagulase	Positive	49	41	83.7	8	16.3
	Negative	11	1	9.1	10	90.9
Pigmentation	Positive	39	34	87.2	5	12.8
	Negative	21	8	38.1	13	61.9
Hemolysin	Positive	42	31	73.8	11	26.2
	Negative	18	11	61.1	7	38.9
Mannite fermentation	Positive	52	41	78.9	11	21.1
	Negative	8	1	12.5	7	87.5

genicity with mucin, the animal virulence test reported upon is proposed as a supplementary method for directly assessing this property in staphylococci since it represents the determination of the capacity of a strain to induce death, which is the acme of virulence.

Summary. Significant enhancement of mouse pathogenicity was observed following intraperitoneal injection of staphylococci with gastric mucin. By means of the adjuvant, the mouse was converted from a host resistant to staphylococcus infection by this route into one readily susceptible with 37 strains. High to fair degree of correlation, depending upon the test, was found between the ***in vitro*** presumptive biochemical tests and intraperitoneal mouse pathogenicity with mucin but some discrepancies were noted between both. The procedure under consideration is proposed as a supplementary method for the direct determination of virulence of staphylococci.

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Comparison of Continuous and Strip Paper Electrophoresis Technics for Study of Serum Glycoproteins.* (22658)

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Paper strip electrophoretic technics are now widely used for fractionation studies of serum proteins. Application of the Hotchkiss-McManus technics for tissue polysaccharides to paper strips by Koiw and Gronwall(1) allows studies of carbohydrate components of the serum proteins. Technics for continuous paper electrophoresis apparatus using vertical curtains have been described by Grassmann and Hannig(2) and by Durrum(3). The use of such equipment allows the collection of serum fractions in quantities which make possible chemical analysis for the glycoprotein and protein components. The work described here was undertaken to compare results obtained by continuous electrophoresis technics with the less tedious paper strip methods.

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