inhibiting metabolism and inactivation of adrenaline. A further possibility is that BAL increases the release or decreases the metabolism of glucagon. The influence of BAL on the metabolism of glucagon and the catecholamines deserves investigation.

Summary. In rabbits, doses of BAL which had no significant influence on blood glucose levels caused marked hyperglycemia when injected at the same time or 30 minutes before injection of a hypoglycemic dose of insulin. Possible explanations of this effect are considered.

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Interdependence of Hyaluronic Acid and M Protein in Streptococcal Aerosol Infections in Mice.*† (25659)

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Coburn *et al.*(1) observed that different streptococcal strains administered by aerosol vary in capacity to infect normal mice. Johnson and Furrer(2) confirmed this observation, and also reported that ability of a strain to produce certain levels of hyaluronic acid (HA) appeared related to capacity of the strain to induce aerosol infection. This report stimulated further investigation into the quantitative role of HA production in this phenomenon in presence and absence of M protein, the substance primarily associated with streptococcal virulence. An opportunity to study the latter aspect of the problem was afforded when several strains of group A streptococci were encountered which colonially resembled the aerosol infective organism but which failed to possess M protein as evidenced by serological analysis.

Methods. Streptococcal strains. Twelve typable group A strains exhibiting various kinds of colonial morphology (4 glossy, 2 matt, 5 muco-matt and 1 mucoid) and the 4 non-typable group A strains which colonially resembled the other mucoid strains were all isolated from Navy recruits. *Aerosol technic.* Details regarding construction of the aerosol chamber, culture and preparations of the cell suspensions, spraying technic and reproducibility of the results produced by this technic have been described(2) and are briefly described here. Each streptococcal strain was incubated in Todd-Hewitt broth for 16 hours at 37°C. After centrifugation, the cells were washed 3 times in Gel-Locke solution and final concentration adjusted to a standard optical density value. Each group of 12 Swiss albino mice was sprayed with a streptococcal suspension twice daily for 3 days, then removed from the aerosol chamber, a throat culture taken,

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TABLE I. Effect of Hyaluronic Acid Produced by Group A Streptococcal Strains on Infectivity in Mice by Aerosol.

M type	Colonial morphology	HA production, mg/ml*	% mouse infectivity†
5	Glossy	.0	0 (12)‡
12	"	.0	0 (12)
13	"	.0	0 (12)
1	Matt	.020	41 (12)
3	Muco-matt	.080	75 (24)
3	Matt	.118	67 (12)
6	Glossy	.132	17 (12)
18	Muco-matt	.170	50 (12)
19	"	.188	67 (156)
19	"	.211	" (144)
18	Mucoid	.225	" (12)
1	Muco-matt	.278	100 (12)
NT§	Mucoid	.118	0 (12)
NT	"	.160	0 (12)
NT	"	.165	0 (12)
NT	"	.170	0 (12)

* Avg value of duplicate samples.

† Positive culture from cervical lymph node or lungs of dead and surviving mice.

‡ No. of mice exposed.

§ No reaction with type-specific streptococcal antisera.

and the mice observed for streptococcal death for an additional 10 days. At this time, all survivors were sacrificed and cultured for the strain of beta hemolytic streptococci used in the aerosol. Those mice exhibiting streptococci in cultures of the cervical lymph node were included in calculation of percentage of mice infected found in column 4 of the table.

Hyaluronic acid determination. To correlate more precisely amount of HA produced and mouse infectivity, the quantitative colorimetric method of HA assay described by Pierce *et al.*(3) was employed in place of the acid-serum method of Seastone used in a previous study(2). Broth culture supernatants of each streptococcal strain grown for 16 hours were obtained by centrifugation at 2500 rpm for 15 minutes. Commercially prepared HA, from umbilical cord,‡ was utilized to prepare a standard curve from which HA values for the broth culture supernatants were determined.

Results. Hyaluronic acid production levels and mouse infectivity data from 16 group A streptococcal strains are shown in the table. Among the 12 typable strains only those producing detectable HA exhibited mouse infectivity. A test of a relationship between the data in columns 3 and 4 of the typable strains only revealed a correlation coefficient of .81 and $P<.01$. Although all 4 non-typable strains produced appreciable amounts of HA, none were capable of infecting mice.

Discussion. Our results confirm the previous findings of Johnson and Furrer(2) that HA production appears to be a required component of group A streptococci for induction of mouse infectivity by the aerosol route. In fact, a significant linear relationship between amount of HA production and infectivity was demonstrated.

Isolation of the 4 group A strains which resembled culturally mouse virulent strains but which differed serologically by failing to demonstrate type-specificity presented an opportunity to obtain information on the role of HA production in infectivity independent of the presence of detectable M protein. Of significant interest was the fact that although all 4 strains produce appreciable amounts of HA, none exhibited mouse infectivity. Of equal importance was the behavior of the 3 typable strains which failed to produce HA and to exhibit aerosol infectivity.

Lack of infectivity in these latter strains could be argued to be due to poor M protein development rather than failure to produce HA since it is conceivable that the quantity of M protein present was sufficient to demonstrate type-specificity but not infectivity. However, it has been reported(2) that several streptococcal strains which type and colonially appear to be rich in M protein, but fail to produce sufficient quantities of HA, exhibited no infectivity when given by aerosol. Therefore, with this evidence in addition to that shown here, it appears that aerosol infectivity of group A streptococci in mice depends on capacity of the strain to produce concurrently HA and M protein.

In view of these results and in contrast to the evidence(4) regarding the minor role of HA in virulence *via* the peritoneal route, there is suggested a different function of HA in establishment of the infectious process by these routes. Just what these differences are cannot be ascertained from the data presented. However, it is postulated that in aerosol infections this muco-polysaccharide material may aid the organism in establishing a foot-

‡ Nutritional Biochemicals Corp., Cleveland, O.

hold in the mucosa of the naso-pharynx. It is further reasoned that the M protein is then primarily responsible for the ultimate course of the infection as is the case with streptococcal cells introduced intraperitoneally. Facts tending to support this hypothesis are: a) The dissimilarity of aerosol infectivity and intraperitoneal virulence of a strain as shown by Coburn(5), b) failure of hyaluronidase treatment to appreciably modify virulence of intraperitoneally injected streptococci(4) and c) as shown by this study, the apparent non-infectivity by the aerosol route of streptococci producing hyaluronic acid but not detectable M protein, or of cells producing M protein but not detectable hyaluronic acid.

Recently Foley *et al.*(6) have reported that virulence of group A streptococci was related to resistance to surface phagocytosis and that this anti-phagocytic capacity was determined by the concomitant presence of large hyaluronic acid capsules and M protein production. Enzymatic destruction of either of these substances resulted in a marked and similar increase in phagocytosis of streptococcal cells by rat leukocytes. It was the opinion of these authors that capsular gel, as well as M protein, plays a significant role in streptococcal pathogenicity. The evidence obtained from our experiment supports their opinion.

Lastly, although there seems to be good evidence that production of HA is essential for aerosol infection, the cause for the high level of HA in broth supernatants of certain highly mouse infective strains has yet to be investigated. Experiments are in progress to determine whether formation of HA is inversely related to hyaluronidase produced.

Summary. Of the 16 group A streptococcal strains tested, only 9 exhibiting type-specificity and production of hyaluronic acid possessed capacity to induce aerosol infections in mice. Conversely, the remaining 7 strains which lacked either one or the other of these attributes failed to demonstrate infectivity by aerosol challenge. A statistically significant relationship between HA production and mouse infectivity was observed.

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Specific Liver and Brain Monoamine Oxidase Inhibition by Alkyl- and Arylalkylhydrazines.* (25660)

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Inhibition of the monoamine oxidase (MAO) enzyme of the brain has been demonstrated to result in an increase in brain levels of serotonin (5-hydroxytryptamine) and the catecholamines(1). This increase is believed to be the mechanism by which iproniazid (1 - isonicotinyl - 2 - isopropylhydrazine, Marsilid) exerts its psychic stimulant action. Since these earlier findings with iproniazid other hydrazine derivatives have been investi-

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