other inbred strains under the same experimental conditions, and to see whether F1 males from different strain combinations have the same effect when used as intermediate hosts as C57Bl/6J males. Even more important is the task of localizing the source of the inducing substance, and then characterizing the inducer itself. It should be pointed out that the data obtained in the present study are also consistent with the hypothesis that the gene involved has been derepressed as a consequence of removal from a female environment, rather than induced to function as a consequence of transfer to a male environment. Intermediate male hosts which have been adrenalectomized, gonadectomized, hypophysectomized, or subjected to a combination of these procedures may help to supply answers to some of these questions.

Finally, it may be worth suggesting that antigenic alterations controlled by hormones may play an important role in the pathogenesis of certain human diseases, suspected of being autoimmune in nature, which occur in a population undergoing acute hormonal changes; examples are systemic lupus erythematosus occurring in females just past puberty, and eclampsia occurring in pregnant women.

*Summary.* It has been previously assumed that the "male" isoantigen of mice is determined by a gene located on the Y chromosome. This study seems to indicate that newborn female skin, nourished in a male environment, can be induced to express the male antigen. Hence the expression of this antigen seems to be influenced by the Y chromosome only in its "male-determining" role, and the gene responsible for the production of this antigen may be present in both sexes.

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## Role of β-Receptors in Sympathetic Regulation of Electrolytes in Rat Submaxillary Saliva.* (32603)

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Previous work on rat parotid gland demonstrated that pilocarpine evokes a saliva that has sympathetic-like characteristics and that these characteristics result from pilocarpine stimulation of sympathetic pathways(1). This sympathetic component is inhibited by pro-

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pranolol(1); consequently, beta adrenergic receptors have been inplicated in the response of rat parotid gland to stimulation by sympathetic nerves. The reflection of this adrenergic stimulation was observed with regard to the protein, but not the electrolyte, composition of the saliva. The object of the present investigation was 2-fold: 1st, to establish more directly the role of alpha and beta adrenergic receptors in regulating the composition of sympathetically evoked saliva and, 2nd, to determine if, in another gland, electrolyte composition is modified when adrenergic blocking agents are used with pilocarpine. Accordingly, the composition of submaxillary saliva evoked by electrical stimulation of the sympathetic innervation, or by pilocarpine, and its subsequent modification by adrenergic blocking agents, were investigated.

*Materials and methods.* Adult male rats (Long-Evans, 300-400 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Fine polyethylene tubing (Clay-Adams PE 10) was inserted to a distance of approximately 4 mm into the oral opening of one submaxillary duct to permit collection of submaxillary saliva. The ipsilateral submaxillary gland was exposed and separated from the adherent sublingual gland, the duct of which was ligated. The trachea was cannulated to facilitate management of possible respiratory complications. For sympathetic stimulation, bipolar electrodes were applied to the cervical sympathetic chain or to the superior cervical ganglion. For parasympathetic stimulation, bipolar electrodes were applied to the submaxillary duct just posterior to the level of appearance of the lingual nerve. A Grass SD 5 stimulator was used to deliver pulses of 2-5 v at a frequency of 20/sec and a duration of 5 msec. Saliva was collected by micropipette from the tip of the duct cannula. Na and K in saliva (3 $\mu$ liter samples) were analyzed by flame photometry after dilution to 2.0 ml in deionized distilled water. Cl and $HCO_3$ were determined in 10 $\mu$ liter samples using the Cotlove chloridometer and Natelson microgasometer, respectively. The beta adrenergic blocking agent propranolol (Inderal‡) and the alpha blocking agent phenoxybenzamine (dibenzyline) were administered i.p. in a dose of 5.0 mg/kg for Inderal and 5 or 10 mg/kg for dibenzyline. Atropine sulfate was given i.p. in a dose of 1 mg/kg. Saliva samples were collected from each animal before, and 30 minutes after, injection of the blocking agents. The submaxillary gland was removed and weighed at the end of each experiment, so that salivary flow rates could be calculated as a volume produced per minute for each milligram of gland ($\mu$ liters/min per mg).

TABLE I. Effects of Inderal on the Electrolyte Composition of Rat Submaxillary Saliva Evoked by Electrical Stimulation of the Sympathetic Innervation.

| | Before Inderal (mEq/liter) | After Inderal* (mEq/liter) |
|---|---|---|
| Na | 20.8 ± 3.3 | 23.7 ± 7.9 |
| K | 149.0 ± 5.9 | 91.3 ± 4.8† |
| Cl | 35.5 ± 4.1 | 62.4 ± 7.9† |
| $HCO_3$ | 90.5 ± 11.2 | 41.3 ± 4.8† |
| Flow | .022 ± .003‡ | .018 ± .002‡ |

Values (M ± SE) are from 6 rats.
* Dose: 5 mg/kg body wt.
† P (before and after Inderal) <.01.
‡ Units for flow are $\mu$liters/mg per min.

*Results.* Electrical stimulation of the cervical sympathetic chain or the superior cervical ganglion evoked a secretion which generally decreased rapidly in flow rate after an initial burst. Variation of the parameters of the stimulus did not result in elevation of the average rate of flow of the sympathetic submaxillary saliva. The electrolyte composition, as well as the average flow rate, of the sympathetic saliva (Table I) differed markedly from that of parasympathetically evoked saliva, as shown by comparison of the data of Table I with Tables II and III. In particular, [K], at 149 ± 5.9 mEq/liter (mean ± standard error), and [$HCO_3$] at 90.5 ± 11.2 mEq/liter, were much higher, and flow rate, at .022 ± .003 $\mu$ liters/min per mg, was much lower for sympathetic than for parasympathetic saliva, while [Cl], at approximately 35 mEq/liter was similar in the two. Differences in [Na] were equivocal. Administration of Inderal (5 mg/kg, i.p.) 30 minutes prior to electrical stimulation of the sympathetic innervation resulted in a marked reduction ($P<.01$) of salivary [K] and [$HCO_3$] (respectively to 91.3 ± 4.8 and 41.3

‡ Inderal was obtained through the courtesy of Dr. Sahagian-Edwards of Ayerst Laboratories, N. Y.

TABLE II. Effects of Inderal on the Electrolyte Composition of Rat Submaxillary Saliva Evoked by Pilocarpine.

| | Before Inderal (mEq/liter) | After Inderal* (mEq/liter) |
|---|---|---|
| Na | 7.3 ± .9 | 8.9 ± 1.3 |
| K | 57.5 ± 4.4 | 40.7 ± 2.7† |
| Flow | .080 ± .005‡ | .073 ± .005‡ |

Values (M ± SE) are from 6 rats.
* Dose: 5 mg/kg.
† P (before and after Inderal) <.01.
‡ Units for flow are μliters/mg per min.

± 4.8 mEq/liter), as shown in Table I. [Cl], however, was elevated (to 62.4 ± 7.9 mEq/liter) after Inderal (P<.01), while [Na] and flow rate were not significantly (P>.05) affected. Administration of dibenzyline in a dose of 5-10 mg/kg resulted in considerable reduction (approximately 70%) in flow rate in each of 6 rats tested. After 10 mg/kg dibenzyline, salivary [K] was reduced by 20-50 mEq/liter in 3 of 5 animals, and unaffected in 2, while [Na] and [Cl] were elevated in each of the 5, by an amount which varied from 10 to 50 mEq/liter. [$HCO_3$] was unaffected.

Stimulation of the submaxillary gland by administration of supramaximal doses (8 mg/kg, i.p.) of pilocarpine resulted in copious flow of saliva. Flow rate (at .080 ± .005 μ liters/min per mg of gland), as well as [K] and [Na] (respectively at 57.5 ± 4.4 and 7.3 ± 0.9 mEq/liter), of the pilocarpine evoked saliva were consistent in magnitude with previously reported values(3-5). Data are shown in Table II. After administration of Inderal (5 mg/kg, i.p.) followed by pilocarpine, salivary [K], which before Inderal had been appreciably lower than levels in sympathetic saliva, declined still further, to 40.7 ± 2.7 mEq/liter. Flow rate of pilocarpine-evoked saliva was probably not (P>.01) affected by Inderal; nor was [Na] (P>.01), as shown by data in Table II.

Isolation of the parasympathetic (chorda tympani) nerve to the submaxillary gland is difficult in the rat. However, it is known(2) that parasympathetic fibers reach the submaxillary gland by coursing along the submaxillary duct. Hence, as an expedient to obtain parasympathetic nerve-evoked saliva for characterization, stimulating electrodes were applied to the submaxillary duct just below the level of the lingual nerve. Stimulation in this manner, using pulses of 2-5 v at a frequency of 20/sec and a duration of 5 msec, resulted in production of a copious flow of saliva. Higher voltages or frequencies did not increase flow rate. Flow rate, at .101 ± .009 μ liters/min per mg, actually exceeded that produced by pilocarpine evoked saliva (Tables II and III). Concentrations of K and $HCO_3$ were considerably lower in parasympathetic than in sympathetic saliva (Table I and III). Administration of Inderal (5 mg/kg, i.p.) 30 min prior to stimulation along the duct did not significantly alter flow rate or the salivary concentrations of Na, K, Cl or $HCO_3$ (P>.01), as shown in Table III. Preliminary experiments with dibenzyline (2 rats) indicate that in a dose of 5 to 10 mg/kg this alpha blocking agent also is without effect on the flow rate or electrolyte composition of saliva evoked by electrical stimulation along the main duct. However, administration of atropine sulfate (1 mg/kg, i.p. to 3 rats) completely blocked this secretion.

*Discussion.* While neural regulation of salivary secretion involves the parasympathetic innervation more generally, involvement of the sympathetic branch is still phylogenetically widespread(3). Both autonomic branches when active, are stimulatory to secretion, but the inorganic composition of the salivas evoked by the 2 branches frequently shows characteristic differences. These differences particularly involve [K] and [$HCO_3$], which are usually appreciably greater in sympathetically than in parasympathetically evoked saliva. High [K] in sympathetic saliva has

TABLE III. Effects of Inderal on the Electrolyte Composition of Rat Submaxillary Saliva Evoked by Electrical Stimulation of Nerves (Chorda Tympani) in the Main Duct.

| | Before Inderal (mEq/liter) | After Inderal* (mEq/liter) |
|---|---|---|
| Na | 9.8 ± 1.3 | 12.3 ± 3.0 |
| K | 46.4 ± 1.9 | 43.0 ± 2.1 |
| Cl | 34.4 ± 1.1 | 32.4 ± 2.4 |
| $HCO_3$ | 28.4 ± 3.3 | 31.6 ± 3.9 |
| Flow | .101 ± .009† | .082 ± .008† |

Values (M ± SE) are from 6 rats.
* Dose: 5 mg/kg.
† Units for flow are μliters/mg per min.

been observed in dog and cat submaxillary (4,5) and in rat parotid(1), while [$HCO_3$] has been observed to be high in sympathetic submaxillary saliva of cat(6). Previous work (7) showed that stimulation of rat submaxillary gland with isoproterenol evokes saliva with exceptionally high [K] (up to 150 mEq/liter) and the present work shows that in saliva evoked by electrical stimulation of the sympathetic innervation [K] is similarly high. In the present experiments, [$HCO_3$] was also measured in sympathetically evoked submaxillary saliva and found to be distinctly elevated over parasympathetic levels.

That beta receptors are involved in the adrenergic response of rat submaxillary gland was implied by early work in which it was demonstrated that a copious secretion resulted from administration of isoproterenol(8) and that this secretion was high in K(7). Evidence was also previously provided that flow of saliva from rat submaxillary gland after epinephrine, while partially suppressed by use of alpha blocking agents, can be additionally suppressed when pronethalol is also administered(9). Present data show clearly that the beta blocking agent Inderal appreciably modifies the composition of saliva evoked by electrical stimulation of the sympathetic nerve. Both [K] and [$HCO_3$] are appreciably and significantly reduced by the administration of Inderal prior to stimulation of the sympathetic innervation. Flow rate, after stimulation of the sympathetic innervation, was not appreciably reduced by Inderal but was reduced by the alpha blocking agent dibenzyline, as previously reported(9). On this basis at least, it appears that alpha as well as beta receptors are involved in the response of rat submaxillary gland to stimulation of the sympathetic nerve.

It has recently been shown that the parasympathomimetic agent pilocarpine may mimic not only effects of parasympathetic nerve stimulation, but also the effects of stimulation of the sympathetic innervation(1). With rat parotid, where this dual effect was demonstrated, only the protein, and not the electrolyte composition, was affected. However, data from the work of McClanahan and Amberson(6) on [$HCO_3$] in dog submaxillary saliva suggested to us that in some instances there may be a sympathetically mediated effect of pilocarpine on electrolytes also. The present data on rat show that [K] of pilocarpine-evoked saliva can be depressed by prior administration of Inderal. Hence it does appear that in some instances the electrolyte composition of saliva also is susceptible to a sympathetic-like action of pilocarpine and that this involves mediation at least by $\beta$-receptor sites. Evidently, the role of the innervation in regulation of the electrolyte and protein composition of saliva can vary among glands even in the same animal.

The electrolyte composition and flow rate of the saliva obtained by electrical stimulation along the main submaxillary duct suggest that this stimulation does not ordinarily spread to sympathetic elements. Flow rate was even higher than is usually observed after pilocarpine, in agreement with observations by Ohlin(10). [K] of duct-stimulated saliva was even lower than after pilocarpine and did not differ significantly ($P > .01$) from [K] of pilocarpine stimulated saliva obtained after Inderal. Duct-stimulated saliva was unchanged in electrolyte composition or flow rate when Inderal was given prior to stimulation, and preliminary observations indicate that dibenzyline is similarly without effect. On the other hand, atropine completely blocked secretion in response to electrical stimulation along the duct. It appears that stimulating parasympathetic innervation by placing electrodes high on the main submaxillary duct can serve as a useful expedient for investigation of parasympathetic regulation of secretion in rat submaxillary gland.

*Summary.* Electrolyte composition ([K], [Na], [Cl], [$HCO_3$]) and flow rate have been characterized in rat submaxillary saliva evoked by electrical stimulation of the sympathetic innervation. [K] and [$HCO_3$] were found to be appreciably higher, and flow rate lower, in sympathetically than in parasympathetically evoked saliva. Administration of the $\beta$-adrenergic blocking agent, Inderal, prior to sympathetic stimulation resulted in significant reduction in salivary [K] of pilocarpine-evoked saliva but did not affect the electrolytes of saliva obtained by stimulation of the parasympathetic innervation. It is concluded that in rat submaxillary gland $\beta$-adrenergic

receptors are involved in regulation of electrolyte composition by sympathetic nerves and by the adrenergic component of the action of pilocarpine.

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## Comparison of HI and HAdI Antibody Response of Military Recruits to Monovalent Vaccines.* (32604)

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The development of efficient and reliable methods for the evaluation of vaccine strains has been an area of continued study by the Virus Laboratory, School of Public Health, University of Michigan. Accumulated experience has led to the thesis that the most pertinent assessment of the relation of strain variation to vaccine protection can be obtained by a comparison of the hemagglutination inhibiting (HI) antibody response of humans given monovalent vaccine containing either the new variant or the previously accepted vaccine strain.

It has been shown by others that the hemadsorption inhibition (HAdI) test, which measures neutralizing antibody is more sensitive and specific than is HI for demonstrating antigenic differences between strains of influenza viruses when sera from lightly immunized mice or chickens are employed(1, 2). Moreover, it has been suggested that such findings might be used as a guide for decisions concerning strain substitution in influenza virus vaccines when antigenic variation is recognized(1).

To evaluate this suggestion a comparison was made of the antibody response of humans to vaccination with monovalent Asian influenza vaccines using HI and HAdI procedures. The vaccines given contained the most widely divergent antigenic prototypes available at the time of the study. The findings comprise the subject of this report.

*Materials and methods. Subjects, vaccines, and bleeding schedules.* In the Spring of 1963 two groups of 22 military recruits received 1 ml of aqueous monovalent influenza virus vaccine containing 200 CCA units of either $A_2$/Japan/305/57 or $A_2$/Japan/170/62 virus. The vaccines were made on special order by a commercial pharmaceutical firm. The subjects were bled before and 2 weeks after vaccination. The sera were separated and stored at 4°C until used.

*Viruses.* The viruses used for testing were from the files of the Strain Study Center, Commission on Influenza, Armed Forces Epidemiological Board at the School of Public Health, Virus Laboratory, Department of Epidemiology, University of Michigan, Ann Arbor, Michigan.

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