(10), in which palpable tumors were obtained in the first passage after 280-430 days.

Summary. Isologous pituitary glands transplanted beneath the kidney capsule of normal hosts were transformed after a long latent period into pituitary tumors. Transplantation bioassays of these tumors showed that they were all mammotropic pituitary tumors exerting some somatotropic effects also.

1. Gorbman, A., Edelman, A., Proc. Soc. Exp. Biol. and Med., 1952, v81, 348.

2. Mühlbock, O., Boot, L. M., Cancer Res., 1959, v19, 402.

3. Bardin, W., Liebelt, A. G., Proc. Am. Assn. Can. Res., 1960, v3, 7.

4. Heston, W. E., J. Nat. Cancer Inst., 1964, v32, 947.

5. Everett, J. W., Endocrinology, 1954, v54, 685.

6. Furth, J., Recent Progr. Hormone Res., 1955, v11, 221.

7. Haran-Ghera, N., Acta Inter. Con. Cancer, 1962, v18, 207.

8. ———, ibid., 1963, v19, 765.

9. Furth, J., Kim, U., Clifton, K. H., Nat. Cancer Inst. Monogr., 1960, No. 2, 149.

10. Furth, J., Gadsen, E. L., Clifton, K. H., Anderson, E., Cancer Res., 1956, v16, 600.

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Modification of the Action of Pilocarpine by Adrenergic Blocking Agents.* (30495)

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Pilocarpine has long been used as a substitute for parasympathetic nerve stimulation, especially of the salivary glands. Recently it was shown that, in the rat, the protein composition (total proteins and amylase) of pilocarpine-evoked parotid saliva differs markedly from that of saliva elaborated in response to stimulation of the parasympathetic nerve(1). On the other hand, amylase or total-protein levels of pilocarpine-evoked saliva are markedly similar to those levels observed when sympathomimetic stimulation of the gland is employed(2). These facts suggest that pilocarpine may be acting on non-cholinergic sites as well as cholinergic sites. Accordingly in the present investigations, the 2 adrenergic blocking agents, 1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride (Inderal),† which blocks adrenergic beta-receptors(3), and phenoxybenzamine (dibenzyline), which blocks adrenergic alpha receptors(4), were used in conjunction with pilocarpine in an attempt to delineate the mechanism of pilocarpine action.

Materials and methods. Adult male Long-Evans rats were fasted (water *ad lib*) for 24 hours prior to experimentation. Rats were anesthetized with Nembutal and the auriculotemporal nerve and the duct from the parotid gland were exposed. The parotid duct was cut and saliva was collected by micro-pipette applied to the duct orifice. Flow of saliva was evoked by electrical stimulation with supramaximal shocks (Harvard stimulator, Model 935B) applied to the auriculo-temporal nerve, or by intraperitoneal injection of supramaximal doses of pilocarpine (1 mg/100 g body weight). When the adrenergic blocking agents, dibenzyline and Inderal were used, they were administered by intraperitoneal injection prior to nerve stimulation or pilocarpine administration. When used in conjunction with pilocarpine, at least equi-molar doses of the blocking agents were injected, and frequently the molar doses of Inderal and dibenzyline were 2 to 4 times those of pilocarpine. Amylase activity of saliva was determined by the method of Myers, Free and Rosinski(5,2), and expressed as milligrams of reducing substance (as glucose) per mg of secretion, formed in a 15-minute digestion

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TABLE I. Effects of Inderal (IN) and Dibenzyline (DI) on Amylase Levels of Rat Parotid Secretion Evoked by Pilocarpine or Auriculo-Temporal (A-T) Stimulation.

Mode of stimulation	Blocking agent	No. of rats	Amylase level* in saliva (mean ± S.E.)
A-T nerve	None	7	31 ± 7.1
	IN	5	39 ± 4.7
	DI	4	38 ± 3.9
Pilocarpine	None	7	235 ± 9.2
	IN	11	38 ± 4.2
	DI	9	374 ±20.0
	IN + DI	6	34 ± 5.8

* Amylase is expressed as mg of reducing substance (as glucose) per mg of saliva formed in a 15-min digestion period at 37°C. Samples of saliva collected in the first 1-3 min of flow following stimulation.

period at 37°C. Glands were removed and weighed and flow rates were then expressed as milligrams of fluid formed per minute per milligram of wet weight of gland.

Results. The amylase levels of rat parotid saliva following stimulation of the gland with supramaximal doses of pilocarpine ranged from 190 to 274 mg/mg of saliva in the initial period of collection and were from 5 to 20 times the levels observed when stimulation of the gland was effected by way of the parasympathetic innervation (range 13 to 65 mg/mg), as shown in Table I. This result is consistent with previous observations (1). When Inderal was administered to the animal preceding stimulation of the gland with pilocarpine, the levels of amylase in this pilocarpine-evoked saliva were markedly reduced (mean 38±4.2 for 11 rats) and closely approximated the mean levels observed when auriculo-temporal stimulation alone was employed to evoke secretion (Table I). On the other hand, when dibenzyline was administered to the rat prior to pilocarpine stimulation of the gland, amylase levels were at least as high as levels of amylase after pilocarpine alone and frequently increased to double those levels observed when pilocarpine alone was used to evoke secretion (Table I). Simultaneous administration of both adrenergic blocking agents, prior to pilocarpine stimulation, did not appreciably modify amylase levels from those observed when Inderal alone was used in conjunction with pilocarpine. It is also apparent that neither Inderal nor dibenzyline modified the effects of auriculo-temporal stimulation (Table I).

Flow rates of parotid saliva evoked by pilocarpine or auriculo-temporal stimulation ranged from .03 to .12 mg/min/mg; with pilocarpine, flow rates were usually in the range .04-.06 mg/min/mg, while with auriculo-temporal stimulation, they fell in a slightly higher range (.05-.09 mg/min/mg). When Inderal or dibenzyline was used in conjunction with pilocarpine, flow rates were somewhat reduced in each case from those observed with pilocarpine alone, and ranged from .023 to .05 mg/min/mg when Inderal was used (mean, 9 rats, .032) and from .025 to .05 mg/min/mg when dibenzyline was employed (mean, 6 rats, .027). Amylase activity was not observed to vary appreciably within the range of flow rates recorded in any of the conditions described. Since amylase levels were markedly different with the various conditions, it is apparent that flow rate was essentially without influence in effecting the modification in amylase observed when inhibitors were employed.

Discussion. The present data indicate that the adrenergic blocking agents, Inderal and dibenzyline, can differentially modify the levels of amylase in secretion evoked by pilocarpine stimulation of the parotid gland, and leave unaltered the levels of amylase in secretion evoked by stimulation of the auriculo-temporal nerve. When the beta-blocking agent is used in conjunction with pilocarpine, amylase levels are reduced to levels observed normally with auriculo-temporal stimulation whereas the alpha-blocking agent causes an elevation in amylase above the levels observed with pilocarpine alone. Since the adrenergic blocking agents were without effect when auriculo-temporal stimulation was employed, it is not likely that any appreciable influence of the blocking agents was mediated through inhibition of cholinergic sites (6). Furthermore, flow rate is not believed to have exerted a primary role in bringing about these alterations in amylase characteristics. It is, therefore, suggested that pilocarpine, at least when levels of specific pro-

teins are considered, while having some actions that are parasympathomimetic, may have additional non-cholinergic effects, which involve stimulation of adrenergic alpha- and beta-receptor sites. The precise locus of action of these adrenergic blocking agents, with regard to their alteration of the action of pilocarpine, remains to be explored.

Summary. Pilocarpine is not an equivalent substitute for parasympathetic nerve stimulation of the rat parotid gland when protein composition of the secretion is considered. Levels of amylase for example are appreciably higher with pilocarpine than with auriculo-temporal nerve stimulation. It has been found that Inderal, an agent which blocks adrenergic beta receptors, reduces amylase levels of pilocarpine-evoked saliva to levels observed with auriculo-temporal stimulation; on the other hand, Inderal does not modify amylase levels observed with auriculo-temporal stimulation. It is concluded that pilocarpine while having some actions that are parasympathomimetic has additional effects which involve stimulation of adrenergic receptor sites.

1. Schneyer, C. A., Hall, H. D., Am. J. Physiol., 1965, v209, 484.

2. Schneyer, C. A., ibid., 1962, v203, 232.

3. Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H., Dornhorst, A. C., Lancet, 1964, v1, 1080.

4. Ahlquist, R. P., Am. J. Physiol., 1948, v153, 586.

5. Myers, V. C., Free, A. H., Rosinski, E E., J. Biol. Chem., 1944, v154, 39.

6. Boyd, H., Burnstock, G., Campbell, G., Jowett, A., O'Shea, J., Wood, M., Brit. J. Pharmacol. and Chemother., 1963, v20, 418.

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Platelet Aggregation and Release of ADP, Serotonin and Histamine Associated with Phagocytosis of Antigen-Antibody Complexes.* (30496)

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Platelet aggregation is a well-recognized phenomenon in anaphylaxis, the Arthus reaction, thrombosis and blood coagulation(1,2, 3). *In vitro* studies with rabbit(4), guinea pig(5,6), pig(7) and human(7,8,9) platelet-rich plasma or platelet suspensions demonstrated that addition of antigen-antibody (Ag-Ab) complexes induced platelet aggregation. This platelet aggregation is associated with release of histamine and serotonin (5-hydroxytryptamine, 5-HT), both *in vivo* (10) and *in vitro*(4,11). Reports concerning the role of complement in platelet aggregation associated with release of pharmacologically active substances are not conclusive(12), and until recently the mechanisms initiating platelet aggregation have not been well understood(12).

Recent evidence has shown that platelets can phagocytose latex (polystyrene) particles. This is associated with release of serotonin and ADP from the platelets, which induces platelet aggregation(13,14). The purpose of the present study was to investigate whether platelet aggregation induced by immune complexes involves a similar mechanism.

Materials and methods. The *in vitro* studies were carried out with human or pig native (no anti-coagulant added) or citrated platelet-rich plasma, or with twice washed platelets (prepared from blood taken into EDTA) suspended in Tyrode-gelatine (0.25% gelatine)(13,14).

The Ag-Ab complexes (BSA-anti-BSA or

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