Accumulation of pp' DDT in certain brain regions of normal and paraoxon treated rats. The concentration of pp' DDT is expressed as μg/g of brain. Acetylcholinesterase activity is expressed as moles of substrate (acetylthiocholine) hydrolyzed/min/g × 10^{-6}. Each group consists of 6 animals

	1 Controls		2 pp'DDT		3 Paraoxon		4 Paraoxon + pp'DDT	
	pp'DDT (μg/g) mean ± SE	Cholin-esterase mean ± SE	pp'DDT (μg/g) mean ± SE	Cholin-esterase mean ± SE	pp'DDT (μg/g) mean ± SE	Cholin-esterase mean ± SE	pp'DDT (μg/g) mean ± SE	Cholin-esterase mean ± SE
Corpus striatum	N.D.	40.51 ± 2.21	2.68 ± 0.04	39.18 ± 1.44	N.D.	11.31[a] ± 0.97	3.01[b] ± 0.05	10.65[a] ± 0.85
Cortex	N.D.	5.95[c] ± 0.51	1.01[d] ± 0.02	6.12[c] ± 0.38	N.D.	3.06[a,c] ± 0.17	1.21[b,d] ± 0.01	2.87[a,c] ± 0.21
Cerebellum	N.D.	34.43[c] ± 1.43	1.77[d] ± 0.03	33.77[c] ± 1.32	N.D.	14.71[a,d] ± 1.21	2.07[b,d] ± 0.04	15.37[a,c] ± 1.02

N.D. = Not detected. [a]Statistically significant difference when compared to corresponding values of group 1 or 2; $p < 0.01$. [b]Significantly different from corresponding values of group 2, $p < 0.05$. [c]Significantly different from the values of brain regions in the same group (1-4), $p < 0.01$. [d]Significantly different from the values of brain regions in the same group (1-4), $p < 0.05$.

of motor function and neurochemical changes or lesions of corpus striatum are associated with certain motor dysfunctions[22-25], it is likely that the accumulation of pp' DDT in this brain region may also be involved in certain toxic effects of pp' DDT. Since the toxic effects of pp' DDT have been reported to be directly related to the concentration of the compound in the brain[5] which is increased by the inhibitor of cholinesterase, it is likely that the central effects of pp' DDT may be enhanced by the prior administration of an organophosphorous compound.

1 pp' DDT (1,1,1-trichloro-2,2-bis(4'-chlorophenyl ethane), used in the above studies was generously supplied by Montrose Chemical Corporation of California, USA.

2 The authors are grateful to Mr M.Z. Hasan for analysis of the samples and to Sandoz, Basel, Switzerland for the gift of paraoxon.

3 K.A. McCully, D.C. Villeneuve, W.P. McKinley, W.E.J. Phillips and M. Hidiroglou, J. Ass. off. agric. Chem. *49*, 966 (1966).
4 W.J. Hayes, Jr, in: DDT, the insecticide dichlorodiphenyl trichloroethane and its significance, vol. 2, p. 100. Birkhäuser, Basel 1959.
5 W.E. Dale, T.B. Gaines, W.J. Hayes, Jr, and G.W. Pearce, Science *142*, 1474 (1963).
6 D.E. Woolley and A.L. Runnells, Toxic. appl. Pharmac. *11*, 389 (1967).
7 W.D. Black and D.J. Ecobichon, Can. J. Physiol. Pharmac. *49*, 45 (1971).
8 A. Sattin, J. Neurochem. *13*, 515 (1966).
9 A. Carlsson, Pharmac. Rev. *11*, 490 (1959).
10 J.H. Quastel, in: Neurochemistry, p. 431. Ch. C. Thomas, Springfield 1962.
11 M.E. Greig and W.C. Holland, Science *110*, 237 (1949).
12 M.E. Greig and J.C. Mayberry, J. Pharmac. *102*, 1 (1951).
13 J. Glowinski and L.L. Iverson, J. Neurochem. *13*, 655 (1966).
14 D.F. Maunder, M.J.H. Egan, E.W. Godly, F.W. Hammond, J. Raburn and J. Thompson, Analyst *89*, 168 (1964).
15 G.L. Ellman, K.D. Courtney, V. Andre, Jr, and R.M. Featherstone, Biochem. Pharmac. *7*, 88 (1961).
16 G.I. Klingman, J.D. Klingman and A. Poliszczuk, J. Neurochem. *15*, 1121 (1968).
17 F. Hobbiger, in: Recent Advances in Pharmacology, 4th edn, p. 281. J & A. Churchill, London 1968.
18 G.J. Maletta and P.S. Timiras, J. Neurochem. *13*, 75 (1966).
19 M.A. Matin, Eur. J. Pharmac. *29*, 168 (1974).
20 V.H. Freed, M.A. Matin, S.C. Fang and P.P. Kar, Eur. J. Pharmac. *35*, 229 (1976).
21 B. Holmstedt, Pharmac. Rev. *11*, 567 (1959).
22 J.D. Connor, G.V. Rossi and W.W. Baker, J. Pharmac. *155*, 545 (1967).
23 G.M. Evertt, L.E. Blockus and J.M. Shepperd, Science *124*, 79 (1956).
24 N.E. Anden, A. Dahlstrom, K. Fuxe and K. Larsson, Acta pharmac. *24*, 263 (1966).
25 A.H. Friedman and G.M. Everett, in: Advances in Pharmacology, vol. 3, p. 83. Academic Press, New York 1964.

An electrophoretic study of proteins secreted by the rat submandibular gland in response to autonomic agonists[1]

S. M. Wilson[2]

University of Durham, Department of Zoology, South Road, Durham DH1 3LE (England), 10 August 1981

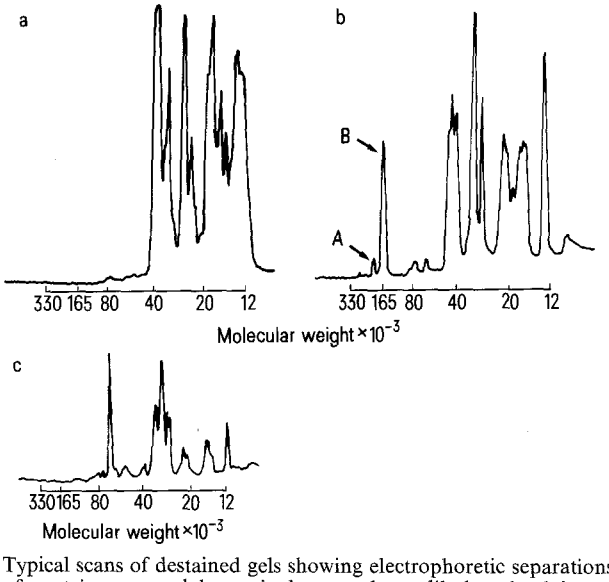
Summary. Proteins secreted by the rat submandibular gland after administration of autonomic agonists have been fractionated by sodium dodecyl sulphate (SDS) - polyacrylamide gel electrophoresis. α-adrenoceptor, β-adrenoceptor and muscarinic agonists were all found to cause the secretion of different protein populations.

In the rat submandibular gland, studies in vivo have shown that activation of α-adrenoceptors, β-adrenoceptors and muscarinic receptors leads to secretion[3]. The composition of the saliva produced may depend upon which receptor is activated. β-Adrenoceptor exitation leads to the production of a saliva characteristically rich in bicarbonate, potassium and protein, with only moderate amounts of sodium[4-6]. Whilst the secretion caused by α-adrenoceptor and muscarinic agonists has lower concentrations of bicarbonate, potassium and protein and more sodium[6,7].

This similarity between the secretory responses to α-adrenoceptor and muscarinic agonists has also been observed in vitro, where both types of agonists caused dispersed rat submandibular acinar cells to release potassium in the absence of any significant effects on protein secretion. β-Adrenoceptor agonists, on the other hand, exert no influence on potassium release but cause the secretion of high molecular weight mucins. The response to both α-adrenoceptor and muscarinic stimuli is dependant upon extracellular calcium, whilst this is not the case for the response to β-adrenoceptor agonists[8,9]. Similarities between α-adrenergic and muscarinic responses have also been observed in electrophysical studies of acini in a number of salivary glands[10–12].

The purpose of the present study is to determine whether the similarity between the effects of α-adrenoceptor and muscarinic agonists is reflected in the types of proteins secreted. To do this salivary protein populations obtained following drug administration were analyzed by SDS - polyacrylamide gel electrophoresis.

Methods and materials. 10 male rats of a Wistar strain were fasted overnight but allowed water ad libitum. The trachea and a femoral vein were cannulated under pentobarbitone anesthesia (Sagatal, 60 mg/kg). Body temperature was maintained at 37–38 °C. A single submandibular duct was exposed below the mylohyoideus and transversus mandibularis muscles, dissected free and cannulated with a short length of polythene tubing (Portex PE10) inserted rostral to the junction of the duct and lingual nerve. α-Adrenoceptors, β-adrenoceptors and muscarinic receptors were stimulated by i.v. injection of phenylephrine HCl, isoprenaline HCl and bethanechol chloride respectively. The doses employed were the smallest consistent with obtaining sufficient material for analysis. Normally, this was 100–200 µg/kg for phenylephrine, 25–50 µg/kg for isoprenaline and 50–100 µg/kg for bethanechol. Saliva was collected from the tip of the ductal cannula using capillary micropipettes (Drummond Microcaps, 50 µl and 20 µl) and promptly transferred to small ice-cold test tubes. In this manner it was possible to collect at least 1 sample for each agonist from every animal. Care was taken to ensure that saliva in the dead space of the duct and the cannula was discarded. Samples were stored at −18 °C pending analysis. In some animals propranolol HCl, tolazoline HCl or dihydroergotamine mesylate were injected i.v.



Typical scans of destained gels showing electrophoretic separations of proteins secreted by a single rat submandibular gland in response to: *a* phenylephrine, 100 µg/kg; *b* isoprenaline, 35 µg/kg; *c* bethanechol, 60 µg/kg.

SDS-polyacrylamide gel electrophoresis was performed on 16×20 cm gel slabs 1.8 mm thick incorporating a 3.3–24% acrylamide concentration gradient, prepared by the method of Thomas[13]. Prior to electrophoresis, sample proteins were treated with 2.5% SDS and 5% 2-mercapto ethanol at 100 °C for 5 min. Gels were stained with Coomassie Brilliant Blue R-250 (0.15% in 25% methanol, 10% acetic acid), and destained by shaking in the same solvent. Molecular weights of sample proteins were estimated by comparing their mobilities with those of a number of protein standards[14,15]. Destained gels were scanned using an Auto Scanner (Helena Laboratories). All drugs and other reagents were obtained from Sigma or Fisons, apart from dihydroergotamine (Sandoz).

Results. Typical scans of destained gels are shown in the figure. Isoprenaline caused the secretion of a complex mixture of polypeptides, including 2 of high mol.wt (fig. bands A and B, 190,000 and 150,000 respectively, although band A was faint and not always observed). At least 6 other bands could be resolved, occupying a range of mol.wt from 12,000 to 47,000.

The α-adrenoceptor agonist phenylephrine caused the secretion of a number of polypeptide species all of which were distinct from those secreted in response to isoprenaline. 8 major bands were observed, ranging in mol.wt from 10,000 to 37,000. The response to phenylephrine was abolished by the α-blocker dihydroergotamine (1 mg/kg), whilst another α-blocker tolazoline (20 mg/kg), although eliminating the response to phenylephrine, caused a very slow continuous secretion. This may be due to an inherent sympathomimetic property of the drug. Propanolol (2 mg/kg) did not influence the response to phenylephrine.

Bethanechol caused the secretion of an extremely dilute saliva and so it was necessary to load more material onto the gels. The protein pattern was found to display considerable variation between individual animals but was always quite distinct from the typical α-pattern and showed a number of similarities to the β-pattern, for example in some animals a small amount of material of 150,000 mol.wt was observed which was indistinguishable from band B of the isoprenaline pattern.

Discussion. Whilst the levels of electrolytes in rat submandibular saliva secreted in response to either α-adrenoceptor or muscarinic agonists are known to be similar[6,7], the present study has shown the populations of secreted proteins to be markedly different. One possible explanation is that more than 1 cell population may contribute to the overall secretion of protein and in this context it is of interest to note that a secretory capacity, mediated by α-adrenoceptors, has been attributed to the convoluted granular tubule of the rat submandibular gland[16].

Whilst phenylephrine acts principally as an α-adrenoceptor agonist[17] it has also been observed to act upon β-adrenoceptors, both in vitro[9] and in vivo[18]. In the present study, both dihydroergotamine and tolazine eliminated the response to the doses of phenylephrine employed, whilst propranolol exerted no influence. Phenylephrine was therefore considered to act exclusively via the α-adrenoceptor.

β-Sympathomimetic stimulation caused the secretion of a number of protein species, including some of high molecular weight, these may correspond to mucins, whose secretion in vitro is mediated by β-adrenoceptors[9]. However, a small amount of this material was sometimes seen when muscarinic receptors were activated, a finding which contrasts with the studies in vitro.

Proteins secreted by the rat submandibular gland have previously been studied by electrophoresis[18,19], but totally

different banding patterns were obtained from those reported here. SDS treatment was not employed and so separation was due partly to the molecular sieving effect of the acrylamide and partly to differences in charge/mass ratio of the sample proteins. Electrophoretic separation of SDS protein complexes on the other hand is due entirely to molecular sieving. Also, previous work employed gels of hich acrylamide concentration, and the large protein species observed in the present study may have failed to enter the gel matrix.

The use of SDS polyarylamide gradient gels has resulted in good resolution of protein species secreted in response to low doses of agonists and has enabled molecular weights to be determined. This in turn could greatly enhance the subsequent identification of protein species.

1 This work is supported by a grant from the Cystic Fibrosis Research Trust, made to Dr C.J. Jones, whose help and supervision is gratefully acknowledged.

2 Reprint requests should be addressed to S.M.W., Department of Physiology Queen Elizabeth College, University of London, Campden Hill Road, Kensington, London LW8 7AH (England).

3 N. Emmelin, J. Holmberg and P. Ohlin, Br. J. Pharmac. *25*, 134 (1965).
4 Y. Yoshida, R.L. Sprecher, C.A. Schneyer and L.H. Scheyer, Proc. Soc. exp. Biol. Med., N.Y. *126*, 912 (1967).
5 J.A. Young and C.J. Martin, Pflügers Arch. *327*, 285 (1971).
6 J.R. Martinez, D.O. Quissell, D.L. Wood and M. Giles, J. Pharmac. exp. Ther. *194*, 384 (1975).
7 J.A. Young, D.I. Cook, G. Jones, J. McGirr and C. Thompson, Aust. J. exp. Biol. med. Sci. *57*, 555 (1979).
8 D.O. Quissell, Am. J. Physiol. *238*, C90 (1980).
9 D.O. Quissell and K.A. Barzen, Am. J. Physiol. *238*, C99 (1980).
10 O.H. Peterson and G.L. Pederson, J. Membrane Biol. *16*, 353 (1974).
11 O.H. Peterson, Physiol. Rev. *56*, 535 (1976).
12 J.A. Young and E.W. van Lennep, in: 'Membrane transport in biology', vol. 4, p. 563. Ed. G. Giebisch. Springer, Berlin, Heidelberg, New York 1979.
13 A. Thomas, Ph.D. Thesis, Univ. of Glasgow, Glasgow 1978.
14 K. Weber and M. Osborn, J. biol. Chem. *244*, 4406 (1969).
15 A.L. Shapiro, E. Vinuela and J.V. Maizel, Biochem. biophys. Res. Commun. *28*, 815 (1967).
16 R.W. Matthews, Archs oral. Biol. *19*, 989 (1974).
17 N. Weiner, in: 'The Pharmacological Basis of Therapeutics', 6th edn, p. 138. Eds A. Goodman Gilman, L.S. Goodman and A. Gilman. Macmillan Publishing Co., New York 1980.
18 K. Abe, K. Yonenda, R. Fujita, Y. Yokota and C. Dawes, J. dent. Res. *59*, 1627 (1980).
19 K. Abe and C. Dawes, Archs oral. Biol. *23*, 367 (1978).

Antigenic differences between a primary hamster lymphosarcoma and its liver metastases

G.V. Sherbet[1], M.S. Lakshmi, G.P. Risely, S.A. Shah[2] and M.E. Tindle

Cancer Research Unit, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP (England), 26 October 1981

Summary. An antiserum was raised in rabbits against a primary metastasizing lymphosarcoma (ML) of the hamster. This was made tumor-specific by absorption with normal hamster tissue extracts. Immunoglobulin-G was prepared and tested for its cytotoxicity towards cells derived from the primary tumor and its liver metastases. The ML-specific IgG was found to be 2-5 times more cytotoxic for cells derived from the primary tumor compared to cells obtained from liver metastases.

Primary tumors are composed of a population of cells which are demonstrably heterogeneous as judged by several criteria of biological behavior, especially metastatic ability[3,4]. Although cells from both non-malignant and malignant tumors may be found in the vascular system, only a very small proportion of these circulating cells survive and successfully form secondary deposits, with the majority being destroyed by the hosts immune system. The metastatic cells have been shown to differ from cells of the primary tumor in susceptibility to drugs, adhesive interactions with different tissues and in their antigenicity[5-7]. Previous investigations carried out in this laboratory on 2 hamster lymphosarcomas have shown that the metastasizing (ML) form is less antigenic than the non-metastasizing (NML) form, and that this is attributable to a loss of antigens from the surface of the ML cells[8]. In this communication, we report our recent findings of antigenic differences between a primary lymphosarcoma in hamsters and its liver metastases.

Materials and methods. Tumors. The metastasizing lymphosarcoma (ML)[9] was maintained by serial transplantation in 2-4-month-old inbred Syrian Cream hamsters. Animals were injected s.c. with 0.2 g of finely chopped tumor tissue in 0.5 ml of medium 199. The biological characteristics of the tumor have been well investigated and its metastatic ability has been previously demonstrated in this laboratory[8,10].

For immunization of rabbits, unicellular suspensions of the tumor were prepared by collagenase disaggregation as described by Guy et al.[11]. Viable tumor cells were separated from non-viable tumor cells and erythrocytes by sedimentation at 1500× g for 15 min in 10 ml of Ficoll 400 (6.35% w/v, Pharmacia)-Hypaque (9.97% w/v, Winthrop Laboratories, Newcastle upon Tyne) as described by Mavligit et al.[12]. Tumor cell suspensions with viability in excess of 95% were used for immunization and in all subsequent procedures of binding and cytotoxicity testing in vitro. Tumor cell content of the preparations was determined as described previously[10] and was approximately 94% for both tumor types.

Target cells. Viable tumor cells obtained from collagenase-mediated disaggregation of primary ML tumors were used as target cells in the cytotoxicity tests. Secondary tumor cells were prepared from livers of the same animals using collagenase as described by Guy et al.[11]. Viable tumor cells were purified from erythrocytes and non-viable cells by flotation on Ficoll-Hypaque discontinuous gradient as described above.

Preparation and purification of antiserum. Half-lop male rabbits (~ 1.8 kg b.wt) were injected every 2 weeks with