mals[5,9]. The increased cell division in the liver graft cannot be interpreted as a general nonspecific stimulatory effect: no such increase was observed in the liver in situ while in the kidney unilateral pneumonectomy seemed to produce a slight decrease in mitotic incidence, possibly due to the stress of the operation. The mitotic stimulation thus appears to be confined to the 'lung field' but within that field any tissue may respond. The fact that results previously obtained for kidney grafts[7] have now been repeated for liver grafts suggests that this may be a general phenomenon.

It is therefore suggested that the compensatory response may be 'field specific' rather than strictly organ- or tissue-specific. This has a bearing on hypotheses for the control of the compensatory response since these are largely based on the concept of organ specificity. Injury to tissue is almost always followed by an increase in the rate of blood flow through homologous tissue and we have suggested that this may stimulate mitosis by causing a change in the local concentration of mitotic regulatory factors[3,7]. This hypothesis could explain the results obtained in the kidney[7] and liver grafts since these shared a common blood supply with the host organ and would hence have been affected by any change in the rate of blood flow following unilateral pneumonectomy.

Increase of blood supply to the liver in situ was not found to stimulate liver growth[10], an observation which may appear inconsistent with our hypothesis. The liver has a system of anastomoses between the larger branches and tributaries of the hepatic artery, portal vein and hepatic vein[11] and diversion of blood via these anastomoses reduces flow through the small vessels of the sinusoids[11,12]. We would suggest that it is the rate of flow of blood through the small vessels, with their intimate contact with the hepatic tissue, that regulates the potential for increased cell division. Because of the system of anastomoses, an increased blood supply to the liver[10] would not necessarily produce in increased flow through the small vessels of the sinusoids.

The rate of cell division in the liver grafts was lower than in the liver in situ, a result consistent with previous results on mitosis and DNA synthesis[13] and growth[14] in ectopic liver grafts. In view of the proximity to the well-vascularized capillary bed of the surrounding lung tissue it seems unlikely that this effect was due to ischemia and the explanation may be found in the absence of normal bile drainage or nerve connections. Stimulation of hepatic cell division was observed in the absence of any injury to the liver graft or to the liver in situ. We consider this inconsistent with the hypotheses[15,16] which propose an organ-specific humoral control system.

Mitotic indices (proportions of mitoses per $10^4$ cells) and SD in organs of Xenopus laevis 2 days after removal of the right lung (unilateral pneumonectomy)

| Organ | Mitotic index Unilateral pneumonectomy | Control |
|---|---|---|
| Left lung | $6.2 \pm 3.7$ ($p < 0.05$) | $2.7 \pm 1.2$ |
| Right lung | $2.2 \pm 1.2$ (NS) | $2.2 \pm 0.3$ |
| Liver graft | $6.6 \pm 1.1$ ($p < 0.001$) | $3.7 \pm 1.0$ |
| Liver | $16.1 \pm 3.5$ (NS) | $14.7 \pm 2.9$ |
| Kidney | $9.5 \pm 2.9$ ($p < 0.05$) | $13.8 \pm 3.0$ |

The number of animals in the unilaterally pneumonectomized group was 7 and in the control group 6. The value for each animal was the mean of 5 sample mitotic counts. Significance of differences (p-values in parentheses) was calculated by Student's t-test with the number of degrees of freedom (11) taken as the number of animals minus 2. For the difference between liver and liver grafts $p < 0.001$.


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## Effect of botulinum toxin on the choline acetyltransferase activity in salivary glands of cats[1]


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*Summary.* The choline acetyltransferase activity of submandibular glands that had previously received a retrograde injection of botulinum toxin via their ducts was found to be markedly lower than in the untreated contralateral glands. In the parotid glands exposed to the same treatment the activity of this enzyme was less affected.


Botulinum toxin causes paralysis of peripheral cholinergic mechanisms, and this is considered to be the consequence of impaired release of acetylcholine from the nerve endings[2]. In the cat, Emmelin[3] showed that injection of the toxin through salivary ducts causes the submandibular and parotid glands to develop within a few weeks a supersensitivity which lasts for several months, similar to that found after parasympathetic denervation. The acetylcholine-synthesizing enzyme, choline acetyltransferase, is confined to the cholinergic nerves, and it was wondered whether the botulinum toxin would have any affect on the activity of this enzyme. Therefore intraductal injections of botulinum toxin have been made into submandibular and parotid glands of cats, and their choline acetyltransferase activity was determined after different time periods. At the end of each experimental period, the sensitivity to chemical stimuli was tested in order to assess the efficiency of the original injection of botulinum toxin.

*Material and methods.* 14 cats of either sex, weighing 2.0–4.4 kg, were used. Under nembutal anaesthesia (36 mg/kg i.p.), either the submandibular duct or the

Table 1. Submandibular glands. Effects of intraductal injection of botulinum toxin on the sensitivity to adrenaline, weight and choline acetyltransferase activity

| Weeks after injection of botulinum toxin | Threshold dose to adrenaline (μg/kg) | | Gland weight (mg) | | | ChAc activity (μg ACh/h/gland) | | |
|---|---|---|---|---|---|---|---|---|
| | Botulinum toxin | Contralateral glands | Botulinum toxin | Contralateral glands | Botulinum toxin percent of contralateral glands | Botulinum toxin | Contralateral glands | Botulinum toxin percent of contralateral glands |
| 1 | 2 | 10 | 585 | 560 | 104 | 61 | 71 | 87 |
| 2 | 1 | 5 | 946 | 1288 | 73 | 76 | 117 | 65 |
| 3 | 1 | 4 | 974 | 1225 | 80 | 75 | 117 | 64 |
| 4 | 1 | 5 | 1179 | 1276 | 92 | 116 | 210 | 55 |
| 6 | 1 | 2 | 525 | 1029 | 51 | 42 | 84 | 50 |
| 6 | 2 | 10 | 722 | 934 | 77 | 97 | 130 | 75 |
| 8 | 1 | 10 | 1145 | 1487 | 77 | 69 | 132 | 52 |
| 16 | 1 | 2 | 856 | 925 | 93 | 135 | 151 | 89 |

parotid duct was cannulated from the mouth. Type A botulinum toxin was used and was derived from an acid precipitate of culture filtrate. The partially purified toxin was freeze-dried and stored in vacuo; 2 μg of this powder dissolved in 0.2 ml of gelatine phosphate buffer pH 6.6 was slowly injected into the duct. The same precautions were taken to prevent secretion as in the study of Emmelin[3]. 1–16 weeks later the animals were anaesthetized with chloralose 40 mg/kg and urethane 600 mg/kg i.p. and the glands were tested for their sensitivity to adrenaline (submandibulars) and methacholine (parotids) as previously described[3]. At the end of the experiments, the glands were dissected out and weighed. Small pieces of the glands were removed for electron and light microscopical examination, using methods previously described[4]. The remainder of the glands was reweighed and rapidly frozen in isopentane cooled by liquid $N_2$ and stored at − 70 °C until used for bioassay of the choline acetyltransferase activity with Hebb's method[5]. The enzyme activity is expressed in μg acetylcholine chloride formed per h per gland; the figures being corrected for the small pieces of tissues taken for the morphological study.

*Results and discussion.* The toxin-treated submandibular and parotid glands showed an increased sensitivity, as judged by the lowered threshold dose for adrenaline and methacholine (see tables 1 and 2) and by the increased amount of saliva secreted in response to larger doses of the drugs, in agreement with previous work[3]. Submandibular glands exposed to the toxin lost weight, while the weight of the parotid glands was unchanged. Similarly, after denervation of these 2 glands[5], the submandibular gland showed the more pronounced fall in weight. The choline acetyltransferase activity in the submandibular glands treated with the toxin was markedly lower than that in the untreated contralateral glands (table 1). In the parotid glands, although the picture was less uniform than in the submandibular glands, most of them had an enzyme activity that was lower on the treated than on the untreated side (table 2). In submandibular glands, there are both pre- and postganglionic nerves on which the botulinum toxin acts[6,7], while in the parotids the cholinergic nerves are only of a postganglionic nature. The fact that the choline acetyltransferase activity in the parotids was less affected by the toxin may suggest that the marked fall in the submandibulars was mainly due to the action of the toxin at a preganglionic level. In this connection, it may be mentioned that the toxin blocks

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Table 2. Parotid glands. Effects of intraductal injection of botulinum toxin on the sensitivity to methacholine, weight and choline acetyltransferase activity

| Weeks after injection of botulinum toxin | Threshold dose to methacholine (μg/kg) | | Gland weight (mg) | | | ChAc activity (μg ACh/h/gland) | | |
|---|---|---|---|---|---|---|---|---|
| | Botulinum toxin | Contralateral glands | Botulinum toxin | Contralateral glands | Botulinum toxin percent of contralateral glands | Botulinum toxin | Contralateral glands | Botulinum toxin percent of contralateral glands |
| 1 | 0.1 | 0.2 | 1468 | 1321 | 111 | 47 | 45 | 104 |
| 2 | 0.1 | 0.2 | 803 | 789 | 102 | 39 | 41 | 95 |
| 4 | 0.5 | 1.0 | 1296 | 1446 | 90 | 27 | 39 | 70 |
| 6 | 0.4 | 0.5 | 1396 | 1421 | 98 | 60 | 58 | 103 |
| 8 | 0.5 | 1.0 | 797 | 803 | 99 | 47 | 63 | 74 |
| 16 | 0.5 | 1.0 | 1171 | 1175 | 100 | 53 | 58 | 92 |

the effect of electrical stimulation of the preganglionic parasympathetic nerves more easily than that of stimulation of the postganglionic nerves in the submandibular glands of cats[7]. The enzyme activity in the toxin-treated submandibulars, expressed as a percentage of the contralateral glands, is of the same magnitude as that reported by Nordenfelt[5] in these glands of cats after previous section of the preganglionic parasympathetic nerves; the mean percentage figure in his study was 60, 2–5 weeks after the surgical procedure. The reduction obtained after the nerve section has been attributed both to degeneration of the preganglionic nerves within the gland and to a fall in the enzyme activity of the postganglionic nerves. Section of the preganglionic parasympathetic nerves to parotids, which have their relay outside the glands, caused the enzyme activity to fall by about 25% in the postganglionic nerves in cats[8] and dogs[9]. In addition, prolonged treatment with a ganglion-blocking drug was followed by a decrease in the enzyme activity also of about 25% in rat parotids[10]. From his finding of a decreased choline acetyltransferase activity in the parotids of cats after cutting the preganglionic parasympathetic nerves, Nordenfelt[8] suggested that the enzyme activity in the postganglionic nerves was dependent on the traffic of impulses in these nerves. This concept is supported by the outcome of a series of experiments, mainly on salivary glands[11]. The profound fall in the choline acetyltransferase activity of the submandibular glands after treatment with botulinum toxin may thus be explained by a decrease of the enzyme activity in both the pre- and postganglionic parasympathetic nerves of the glands; the decrease in the postganglionic nerves being partly a consequence of a reduction or loss of the propagation of impulses along these nerves resulting from the toxin interfering with the transmission at the ganglia. Morphological examination of the postganglionic nerves within the glands treated with the toxin showed no obvious differences from those in the contralateral glands, except that the density of cholinesterase positive nerves appeared to be greater in those glands showing most atrophy. No clear evidence of axonal sprouting, as previously found in somatic motor nerves after botulinum toxin treatment of skeletal muscles in mice[12,13], has so far been observed. Conversely, in the same sort of skeletal muscle preparation, no change in choline acetyltransferase activity was noticed[14].

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## Reproductive consequences of mega vitamin E supplements in female rats[1]

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*Summary.* Female rats fed 0, 25, 2500 and 10,000 IU vitamin E/kg diet for 3 months were examined for reproductive performance. On 10,000 IU vitamin E/kg diet, the fertility of inseminated rats was significantly reduced as compared to rats given normal or nutritional levels of vitamin E.

Vitamin E supplementation has been proclaimed by some medical practitioners and popular press to be beneficial for health and for protection against numerous ailments. Vitamin E has been advertised widely for self-medication and is available freely without prescription. Even though vitamin E has been established as an essential factor for successful reproduction in the female rat[2], potential hazards of hypervitaminosis E warrant thorough investigation. The study presented in this report demonstrates the harmful effects of excess vitamin E on the fertility of female rats.

*Methods and materials.* Female weanling rats of Wistar strain weighing approximately 50 g were randomly divided into 4 groups with 5 rats in each group. The rats were housed in individual hanging wire-meshed cages in a room which was lighted from 6.00 h to 18.00 h daily and thermostatically maintained at 23 °C. Food and water were provided ad libitum. The basal vitamin E deficient diet of Draper et al.[3], with different levels of vitamin E supplements was fed to the 4 groups of rats for 3 months. 1 group of rats was fed only the basal diet without any vitamin E supplements. 2 of the 4 groups of rats were treated with excess vitamin E, the levels being 2500 and 10,000 IU vitamin E (dl-α-tocopheryl acetate)/kg diet respectively. The rats in the control group were fed 250 IU/kg diet for the first month and then 25 IU/kg diet for the rest of the experimental period. At the end of 3 months, female rats were mated with males of the same age which were maintained on commercial laboratory chow. The fertility rate of males and females on control diets was between 90 and 100% at this stage of life. The mating was carried out by exposing each female rat to a different male rat during each day until insemination occurred or up to a maximum of 10 days (2 estrus cycles). Vaginal swab technique was used to examine estrus. The day of insemination was determined by the appearance of sperms in the vaginal smear. After insemination, all females were housed individually and were killed 19 days post-insemination. The uteri were examined for the number of live, dead and resorbed fetuses and for the implantation sites. All fetuses were examined for possible external malformations.

*Results and discussion.* The effects of dietary vitamin E supplements on reproductive performance of female rats are presented in the table. 4 out of 5 rats in vitamin E-deficient and control groups, and all 5 rats in 2 groups

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