

indicates that more cells were present per unit area of the gland in the female and also an average size of cells was smaller in the gland in the female than in the male. The latter is supported by the finding that the RNA:DNA ratio and the protein:DNA ratio of the gland were much lower in the female than in the male (table 3).

The deprivation of food resulted in a comparable loss of body weight in adult mice of both sexes: 18–19% after 24 h and 25–27% after 48 h (table 1). In the adult male rat, a 25% decrease in the b.wt occurred after 72 h of fasting¹³. This probably implies a faster metabolic rate in the mouse than in the rat.

A 48-h fasting resulted in a 16% reduction in the weight of the submandibular gland in the male and a 31% reduction in the female (table 1). Thus, the ratio of gland weight to b.wt after a 48-h fasting was increased in the male but it was decreased in the female.

The loss of gland weight was accompanied by a comparable loss of the total RNA and protein contents of the glands of both male and female mice. In the male, the percent loss of RNA was 16% and that of protein 18%, while in the female, the former was 34% and the latter 29%. The concentrations of RNA and protein, however, were essentially similar in both sexes before and after fasting (table 2).

The greater biochemical changes in the female gland after fasting may be related to the findings that the submandibular gland of female mice contains a greater proportion of acinar cells than ductal cells^{2,3,18} and that total inanition affects more the acinar portion than the ductal portion of the salivary gland¹³. It is not known if the cellular turnover rates of RNA and protein in the gland vary between

different types of cells or between glands of different sexes. It has been shown, however, that cells from the female gland had a greater oxygen consumption, glucose uptake, aerobic glycolysis and adenosine triphosphate production than cells from the male gland^{10–12}; fasting affects these energy-dependent biochemical processes preferentially.

Besides, the concentration of DNA in the gland was significantly ($p < 0.01$) increased following fasting, particularly in the male mice. This was probably secondary to a loss of some cellular components including RNA, protein and water among others. On the other hand, after a 48-h fasting, the total DNA content did not seem to change in the male, but was decreased 18% in the female. This suggests that some cell loss might have occurred as well in the female gland after fasting.

Table 3. Effect of fasting on the RNA:DNA ratio and the protein:DNA ratio in the submandibular gland of male and female mice

	RNA:DNA	Protein:DNA
Male		
Fed	3.28	73.6
Fasted	2.80	61.6
Female		
Fed	2.64	32.8
Fasted	2.14	28.4

1 This study was supported by the U.S. Public Health Service Research Grant CA 17038. The authors are indebted to Mr I. Borcsanyi for his technical assistance.
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Movements of supernumerary hindlimbs after innervation by single lumbar spinal nerves of *Xenopus laevis*

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Summary. Lumbar spinal nerves S8, S9, and S10, together innervating normal hindlimbs in *Xenopus laevis*, were tested to cause coordinated movements in grafted hindlimbs. It could be shown that this ability is mainly restricted to lumbar nerve S9.

Grafted hindlimbs of the anuran *Xenopus laevis* can only move in coordination and apparent synchrony with the adjacent normal hindlimb (phenomenon of homologous response³ when innervated by branches of lumbar spinal nerves^{4,5}. The same results apply to urodelen amphibia, i.e. *Ambystoma*⁶. Since a hindlimb of *Xenopus* is innervated by the spinal nerves S8, S9, and sometimes S10, the question arises, whether one of these 3 lumbar spinal nerves alone is able to cause adequate limb movement.

Material and methods. Tadpoles of the African clawed toad *Xenopus laevis* were obtained according to the method

described by Andres et al.⁷. Autoplastic transplantations of left hindlimbs adjacent to the normal right hindlimb were performed on tadpoles at development stage 54⁸. The animals were anaesthetized in MS 222 and operated on in Holtfreter solution. One of the lumbar nerves, either S8, S9 or S10 (each in 8 tadpoles) was dissected as closely as possible to the base of the right hindlimb. The central stump of the specific lumbar nerve was deviated to the graft's plane of amputation. The animals were raised at 22±2 °C. When the tadpoles had reached stages 62–66 (34–63 days after the operation), the motility of grafted left

Motility in grafted left hindlimbs caused by deviated lumbar nerves and motility in normal right hindlimbs caused by remaining lumbar nerves

Grafted left hindlimbs				Innervation by deviated lumbar nerve	Normal right hindlimbs				Innervation by: branches grown back from deviated lumbar nerve	Remaining, undissected lumbar nerve(s)
Toads with movements at			Toads with movements at							
hip	knee	ankle	hip		knee	ankle				
1	○	○	○	S8	1	●	●	●	S8	S9 (S10 absent)
3	○	○	○	S8	3	○	●	●	S8	S9 (S10 absent)
2	○	○	○	S8	2	●	●	●	S8	S9 and S10
2	○	○	○	S8	2	○	●	●	S8	S9 and S10
4	○	○	●	S9	4	●	●	●	S9	S8 (S10 absent)
2	○	●	●	S9	2	●	●	●	S9	S8 (S10 absent)
2	○	●	●	S9	2	●	●	●		S8 (S10 absent)
5	○	○	○	S10	5	●	●	●		S8 and S9
3	○	○	●	S10	3	●	●	●		S8 and S9

Visible movements ●. No visible movements, at best muscle contractions ○. Visible movements in normal right hindlimbs caused by branches grown back from deviated lumbar nerve ○.

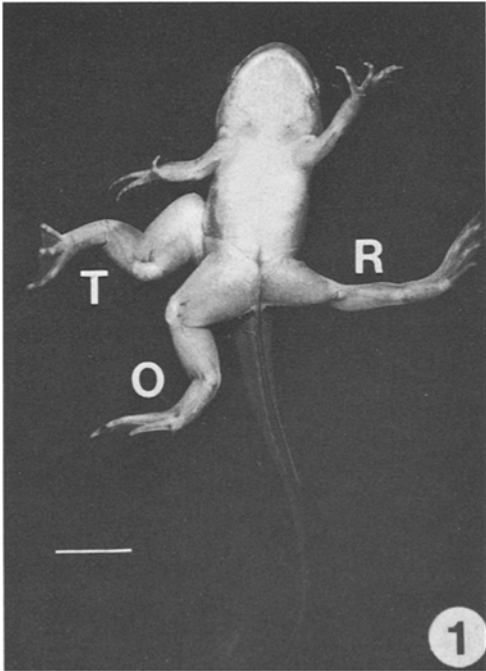


Fig.1. Ventral view. Young toad with grafted left hindlimb (T). Homologous response in knee and ankle joints of grafted and normal right hindlimb (O). Regenerated hindlimb (R). Bar indicates 5 mm.

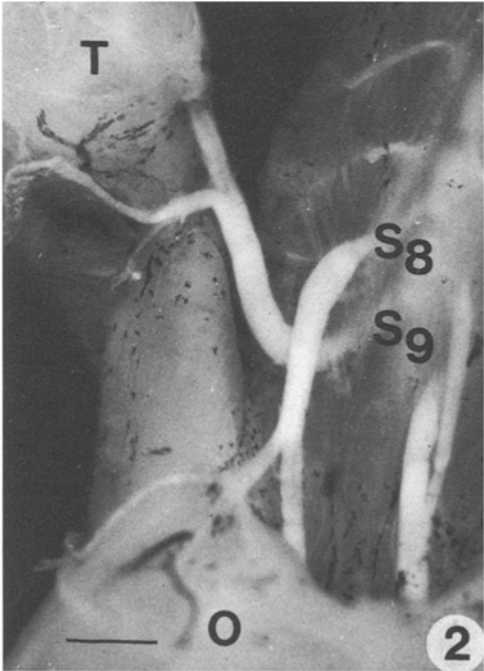


Fig.2. Same animal. Ventral view. Grafted left hindlimb (T) innervated by deviated lumbar nerve S9. Normal hindlimb (O) innervated by remaining lumbar nerve S8 (S10 is absent). There are no branches from S9 to the normal hindlimb. Bar indicates 1 mm.

hindlimbs and normal right hindlimbs was examined with the now young toads by visual inspection in free swimming. The entrance of deviated nerves into the grafts and the innervation of normal hindlimbs by the remaining, undissected lumbar nerves were traced under the dissection-microscope and photographed. Double innervation of normal right hindlimbs by remaining lumbar nerves and by branches of deviated lumbar nerves which had grown back into their original hindlimb, i.e. the ones they had been taken from, was the cause for stimulating-experiments (Grass S4K: square pulses at 1-50/sec, 0.1 msec duration and up to 6 V) in order to detect the influence of branches grown back upon the formerly observed motility.

Results and discussion. The results are summarized in the table. All visible movements in left grafts and right hindlimbs were coordinated swimming-movements, i.e. rhyth-

mic flexions and extensions in hip, knee, and ankle joints. Coordinated movements in corresponding joints of normal and grafted hindlimbs were apparently synchronous (phenomenon of homologous response³). Results clearly showed that the phenomenon of homologous response is mainly restricted to grafts innervated by lumbar nerve S9 (table; figures 1 and 2). Similar experiments have been carried out by Czéh and Székely⁹ who grafted *Ambystoma*-forelimbs and established innervation of the grafts by deviating either the 3rd, 4th or 5th spinal nerve. The authors could not detect any major difference in the movements of the grafted limbs by visual observation: the grafts moved in a coordinated manner and synchronously with the normal limb. Differences in apparent coordination of limb movement could be detected by electromyographic recordings of muscle activity patterns: provided that the 4th

nerve innervated the grafts, muscle activity was close to the normal pattern.

The finding that synchronous, coordinated movements can be produced in 2 adjacent hindlimbs by single but different lumbar spinal nerves without nerve-branches grown back (3 animals with deviated S10; 2 animals with deviated S9, figures 1 and 2), is of particular importance. Anatomically, these 5 cases support the conclusions of Hollyday and Mendell⁴ that in *Xenopus* individual motor neuron pools are able to produce homologous response. Thus innervation of homologous muscles in normal and grafted hindlimbs is not provided by branching of motor neuron axons, a fact which is also seen in animals with branches from deviated nerves contributing to the normal hindlimb's innervation: branches from S9 caused nothing but muscle contractions in the thigh; branches from S8 lead to muscle contractions in the hip (5 cases) and visible movements (flexions) could be detected only in the hip (3 cases).

If we consider coordinated movements of normal hindlimbs, no matter which form of partial innervation by remaining lumbar nerves exists, it is surprising that only grafts innervated by lumbar nerve S8 showed no motility at any joint. This observation, together with previous data on deviated hindlimb nerves in *Xenopus*¹⁰, strongly supports the assumption that the innervated periphery of the grafts is unable to alter the function of the respective spinal centres⁹. The frequent occurrence of coordinated movements after

S9 deviation can possibly only be understood by the assumption of selective mechanisms bringing about specific nerve-muscle connections¹¹. But: 'No evidence is available on the ability of anuran (i.e. *Xenopus* and *Rana*) nerves to selectively innervate or reinnervate particular muscles' (Mendell and Hollyday¹²).

- 1 Acknowledgments. I wish to thank Professor C. Harte and Dr D.K. Hofmann for their support and interest in this work. I am very grateful to Mr R. Kucharek for correcting the English manuscript.
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Effect of pylorus ligation on gastric mucosal mast cell population in normal and adrenalectomised albino rats

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Summary. Pylorus ligation in normal albino rats acts like a stressor leading to degranulation of mast cells in gastric mucosa, thereby decreasing their number. This decrease is less pronounced when pylorus ligation is done in adrenalectomized rats. This implies that action of a stressor on gastric function involves the adrenal steroids which liberate the powerful gastric stimulant histamine from gastric mucosal mast cells.

The interrelationship between adrenal cortex, stress and gastric secretion is well-documented¹⁻³, however, its exact mechanism is not well-understood. Further, it has been postulated that histamine liberators release histamine from locally present mast cells⁴⁻⁶.

The mucosa of rat's glandular stomach has a high mast cell population with profuse histamine content. Humoral⁷ and neural influences⁸ degranulate the mast cells resulting in their losing metachromasia and hence undergoing reduction in number⁹.

The present experiment is planned to study the effect of stress in the form of pylorus ligation on gastric mucosal mast cell population in normal and adrenalectomized albino rats.

Materials and methods. 30 healthy albino rats of either sex, weighing between 100 and 150 g and housed in separate cages, were divided into 3 groups of 10 rats each.

Group 1 served as control. Food and water were given ad libitum for 7 days. Solids were withheld on the 8th day. The animals were sacrificed on the 9th day and their stomachs were removed for histological processing.

Group 2 formed control-Shay group. All the rats were maintained on food and water ad libitum for 7 days followed by total starvation for the next 24 h at the end of which period the rats were subjected to pylorus ligation by

the method of Shay¹⁰. 6 h after the operation the animals were sacrificed and their stomachs were removed for histological processing.

Group 3 formed adrenalectomy-Shay group. All the 10 rats in this group were subjected to bilateral adrenalectomy by the method of Venning. They were maintained on solids and saline ad libitum for 7 days and only on saline for the next 24 h. The rats were then subjected to pylorus ligation as in group 2. 6 h later the animals were sacrificed and the stomachs were removed for histological processing.

The glandular portion of the stomach from each rat was fixed in 4% aqueous solution of basic lead acetate for 48 h. Routine histological procedures followed and 10 µm thick sections were made and stained in 1% aqueous solution of toluidine blue for 1 min. The mast cells in the mucosal layer could be readily identified by the metachromatic purple stain against bluish background. A calibrated ocular micrometer was introduced into the eye piece of the microscope and the mast cells were counted under high power objective and expressed for 1 mm² of the gastric mucosa.

Results. The table shows: 1. Pylorus ligation in normal rats (group 2) has resulted in a highly significant decrease ($p=0.001$ or less) in mast cell population when compared to control rats (group 1). 2. Pylorus ligation in adrenalecto-