different form in adult and infant rats, and that this might be due to a difference in transport of fat and in the part played by the lungs in this process. A preliminary experiment using a 5-day-old puppy showed the same picture as in infant rats.

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Lack of Evidence for a Cholinergic Mechanism in Sympathetic Transmission

THE nerve supply to many sympathetic effector organs contains cholinergic nerve fibres as well as adrenergic fibres. Recently, the hypothesis has been put forward that a cholinergic mechanism is involved in sympathetic transmission[1,2]. It has been suggested that acetylcholine released from cholinergic nerve endings liberates from a local tissue store sympathetic transmitter which in turn acts on the effector organ.

One of the ways in which this hypothesis has been tested has been to use a hemicholinium, HC3 (ref. 3), which inhibits the formation of acetylcholine[4,5]. If sympathetic transmission does depend on the release of acetylcholine the effect of stimulating post-ganglionic sympathetic nerves should be blocked by HC3. Rand and Chang[6] showed that HC3 blocked the contractions of the isolated ductus deferens of the guinea pig produced by stimulation of the sympathetic nerve. Later they showed similar effects on a number of other sympathetic effector organs, including the isolated uterus and colon of the rabbit, the perfused rabbit ear, the isolated atria of the cat and the piloerector muscles of the cat's tail, most of which are known to contain both cholinergic and adrenergic fibres in their nerve supply[7].

To determine whether adrenergic transmission is necessarily mediated via a cholinergic mechanism we have used the nictitating membrane of the cat. This can be set up as an isolated nerve–smooth muscle preparation[8], and the evidence is that under these conditions neuromuscular transmission is purely adrenergic[8]. The post-ganglionic nerve fibres were stimulated with supramaximal square-wave shocks of 0·5 msec. duration at frequencies of 5, 10, 20, 25, 50 and 100 c./s. for periods of 7 sec. repeated every minute. HC3 in concentrations of 20–540 μgm./ml. ($=3 \cdot 5 \times 10^{-5} - 9 \cdot 4 \times 10^{-4}$ M) had no effect on the contractions of the muscle in response to nerve stimulation; even when stimulation was carried out in this way for more than 1 hr., there was no effect. These findings confirm those reported by Wilson and Long[9], who tested the action of HC3 on the nictitating membrane *in vivo*.

HC3 prevents the formation of acetylcholine not by inhibiting choline acetylase directly but by preventing the passage of choline through cellular membranes which enclose the enzyme[5]. The blocking effect of HC3 may not therefore become apparent unless the duration and frequency of nerve stimulation are sufficient to deplete the stores of acetylcholine[4]. In our experiments the higher frequencies of intermittent stimulation were greater than those which were required to produce an effect on the test objects used by Chang and Rand[7]. Nevertheless, the possibility remained that HC3 would not block the contractions of the nictitating membrane unless the nerve were stimulated continuously. When this was done at frequencies of 25 c./s. and 100 c./s. the contraction produced began to wane almost immediately, but the presence of HC3 in concentrations of 60–100 μgm./ml. ($=1 \cdot 05 \times 10^{-4}$–$1 \cdot 75 \times 10^{-4}$ M) did not alter the rate at which the contraction decayed, even when HC3 was present for as long as 40 min.

Another indication that transmission is cholinergic might be that choline prevents or reverses the waning contraction produced by continuous stimulation at high frequencies. It was found, however, that choline in concentrations of 1·4–200 μgm./ml. ($=10^{-5}$–$1 \cdot 42 \times 10^{-3}$ M) did not diminish the rate of decay.

These results do not support the suggestion that a cholinergic mechanism is concerned in sympathetic transmission over adrenergic nerve fibres.

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HISTOCHEMISTRY

Leucine Aminopeptidase in the Mammary Gland of the Cow and Rat

MORPHOLOGICALLY the mammary gland is a cutaneous gland, but physiologically it must be classed as an accessory gland of the reproductive system. In the various stages of the life of the female mammals the mammary gland undergoes many structural changes. Up to the age of puberty it is rudimentary in structure. During pregnancy the endocrine factors furnish a stimulus for proliferation of the mammary tissue, resulting in a complete transformation and maturation of the gland. The appearance of fine granulations and fat droplets in the epithelium cells indicates the onset of secretion in advanced pregnancy.

To our knowledge, the activity of the histochemically demonstrable leucine aminopeptidase in the mammary tissue has not been studied previously; therefore the object of our present work was to investigate the leucine aminopeptidase activity in the mammary gland of the cow and rat in the various stages of development.