measurement 1.9 ± 0.2 mμmoles, the variance ratio being statistically significant at the 5% level.

1 mμmole of phosphorus corresponds approximately to the removal of a 20 Å layer of enamel. The results suggest that the outer 50 Å of enamel is more heterogeneous than the underlying enamel. This may be due in part to the difficulty in controlling the removal of superficial organic matter by polishing.

Résumé. On a développé un ultramicrodosage du phosphore et a appliqué ceci à l'extrait du papier à filtrer qu'on a saturé du tampon critique à pH 3,5 et tenu sur la surface d'une dent dans la bouche.

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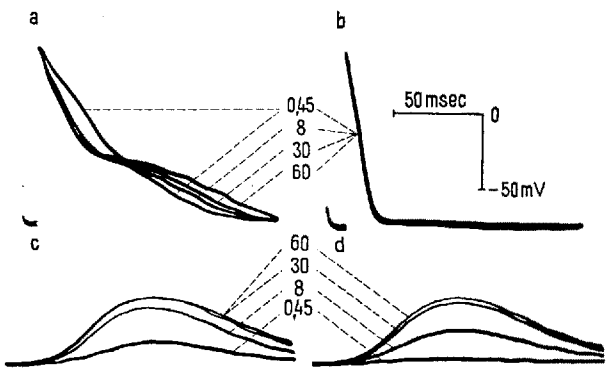
Effets de l'acétylcholine et de la fréquence de stimulation sur le potentiel d'action et sur la contraction de l'oreillette gauche du rat

L'effet inotrope de l'acétylcholine (ACh) sur le myocarde dépend de la fréquence des contractions. Ainsi, l'oreillette gauche du cœur de rat, stimulée *in vitro* à la fréquence de 2,4/sec, est plus fortement inhibée par l'ACh qu'à la fréquence de 0,1/sec[1]; l'action de l'ACh sur les contractions de fréquence encore plus basse est faible ou nulle.

VAUGHAN WILLIAMS[2] a montré que le potentiel d'action des oreillettes du lapin est toujours modifié sous l'action de l'ACh, même après un arrêt de longue durée, alors que la force de la contraction de reprise est relativement grande. Il constate, dans un travail ultérieur[3], l'existence d'une corrélation entre la force de contraction et la durée d'un post-potentiel.

Nous avons examiné les effets de l'ACh sur la force de contraction et sur le potentiel d'action intracellulaire de l'oreillette gauche isolée du rat, préparée selon une technique décrite précédemment[1]. La préparation a été stimulée électriquement à la fréquence de 2,2/sec. Les répercussions de divers temps d'arrêt sur la contraction de reprise et sur le potentiel d'action ont été étudiées sans et avec adjonction d'ACh (Figure).



Effets des changements de l'intervalle entre les stimulations sur le potentiel d'action intracellulaire (enregistrements a et b) et sur la force de contraction (enregistrements c et d) de l'oreillette gauche du rat. (a), (c) sans acétylcholine, (b), (d) avec acétylcholine 10^{-5} *M*. Les chiffres indiquent, en secondes, la durée de l'intervalle.

(a) *Sans ACh.* Un arrêt de 8 sec a fortement accéléré la phase initiale de la repolarisation. Une prolongation de l'arrêt a encore accentué cet effet, le maximum étant atteint à 30 sec environ. En revanche, la phase terminale de la repolarisation a été ralentie par l'arrêt: sa courbe, qui a présenté une concavité vers le haut lorsque la préparation était stimulée à la fréquence de 2,2/sec, est devenue convexe après un arrêt de 8 sec; cet effet a augmenté lors d'une prolongation de l'arrêt au-delà de 30 sec alors que la force de contraction qui s'était également accrue avait déjà atteint son maximum.

(b) *Avec acétylcholine à 10^{-5} M.* L'ACh a diminué la force de contraction et raccourci la durée du potentiel d'action de l'oreillette stimulée à la fréquence de 2,2/sec. Cependant, après 1 min d'arrêt, la force ainsi que la durée de la contraction de reprise ont été identiques avec et sans ACh.

L'ACh n'a donc pas agi sur la contraction qui suit un arrêt suffisamment long bien qu'elle ait raccourci le potentiel d'action. Quelle qu'ait été la durée de l'arrêt – fût-elle même de 5 min – la repolarisation a été accélérée par l'ACh.

Ces expériences suggèrent que l'effet de l'ACh sur la force de contraction de l'oreillette du rat n'est pas lié aux modifications du potentiel d'action qu'elle provoque. En effet, la contraction qui suit un arrêt suffisamment long n'est pas inhibée par l'ACh 10^{-5} *M* bien que le potentiel d'action subisse le raccourcissement caractéristique).

Summary. The force of contraction of the isolated left auricle of the rat could not be correlated to the duration of the intracellular action potential, if stimuli were spaced at intervals of 30 sec or more. With these long intervals, acetylcholine had no inotropic effect but still shortened the action potential.

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Institut de Physiologie de l'Université de Genève (Suisse), le 12 septembre 1961.

[1] F. BAUMANN, L. GIRARDIER et J. POSTERNAK, Helv. physiol. Acta *18*, 509 (1960).
[2] E. M. VAUGHAN WILLIAMS, J. Physiol. *147*, 325 (1959).
[3] E. M. VAUGHAN WILLIAMS, J. Physiol. *149*, 78 (1959).
[4] Ce travail a bénéficié d'un subside de la Fondation EMIL BARELL.

A Possible Role for Histamine in Larval Growth

There are some 25 known biological reactions where the enzyme decarboxylase is needed. Of these reactions, at least 9 are involved in the transformation of an immediate precursor into an active amine[1]. In this group there are important biologically active diamines such as histamine and serotonine which are decarboxylase-dependent for their synthesis. The co-enzyme of decarboxylase is pyridoxine and the absence of this vitamin in the diet

[1] W. CLARK, Circ. Res. *9*, 721 (1961).

results in decarboxylase inactivation[2]. Similarly substances which compete with pyridoxine inhibit decarboxylase[3]. These substances have a much faster action than depriving the organism of pyridoxine[4,5]. Unfortunately these inhibitors such as semicarbazide or deoxypyridoxine, lack specificity and have limited value in assessing the role of various decarboxylase-depending substances on different physiological mechanisms. However, in dealing with diamines advantage can be taken of the fact that these substances are transformed into relatively inactive products by various enzymes including diamineoxydase (histaminase) which is inhibited by aminoguanidine[6,7]. By using these inhibitors, KAHLSON and his group were able after many elaborate and well coordinated studies, to attribute to histamine a seemingly direct role for all mammalian growth processes[8]. KAHLSON and ROSENGREN extended these studies to vegetables and their results also favour the importance of histamine in growth.

Knowing the vital importance of histidine[10], the immediate precursor of histamine, and also of pyridoxine in larval growth of *Tribolium confusum* we investigated the effect of alterations in histamine metabolism on this phenomenon.

Experimental Procedure. The animal used was *Tribolium confusum*. The basal diet was composed of whole wheat flour with 5% dried brewer's yeast[11]. For all substances studied 30 larvae were used. These larvae were divided into 3 groups kept at a temperature of 28 ± 1° C and relative humidity of 70 ± 5%. The number of larvae transformed into pupae were counted starting on the 16th day. Five experiments were done simultaneously.

In experiment 1, six groups were used with respectively 0.001%, 0.01%, 0.1%, 2% and 4% aminoguanidine added to the diet; in experiment 2, four groups were used with respectively 0.01%, 1%, 1% and 2% of semicarbazide added to the diet; in experiment 3, four groups were used with respectively 0.01%, 0.1%, 1% and 2% deoxypyridoxine added to the diet; in experiment 4, two groups were used with 0.1% aminoguanidine and 0.1% semicarbazide together in the first group and 0.1% aminoguanide and 0.1% deoxypyridoxine together in the second group; in experiment 5, one group was used with 0.005% semicarbazide and 0.005% deoxypyridoxine added together to the diet.

Results. Experiment 1: *Effect of aminoguanidine on larval development.* It is possible with this substance to completely inhibit the activity of diamine oxydase in mammals. It is shown in Figure 1 that 4% of aminoguanidine in the diet greatly retards growth without being lethal. Furthermore concentrations of this substance 400 times smaller, that is 0.01%, have a highly significant effect on growth. Consequently aminoguanidine markedly inhibits larval development but is non-toxic.

[2] W. H. SEBRELL and R. S. HARRIS, *The Vitamins*, vol. III (Academic Press, 1954).
[3] W. W. UMBREIT and J. G. WADDELL, Proc. Soc. exp. Biol. Med. *70*, 293 (1949).
[4] R. E. PARKS JR., G. W. KIDDER, and V. C. DEWEY, Proc. Soc. exp. Biol. Med. *79*, 287 (1952).
[5] R. J. WILLIAMS, R. E. EAKIN, E. BEERSTECHER, and *X*. SHIVEW, *The Biochemistry of the B Vitamins* (Reinhold, New York 1950).
[6] E. A. ZELLER, Helv. chim. Acta *21*, 880 (1938).
[7] W. SCHULER, Exper. *8*, 230 (1952).
[8] G. KAHLSON, Lancet *1960*, 68.
[9] G. KAHLSON and E. ROSENGREEN, J. Physiol. *154*, 2 (1960).
[10] A. LEMONDE and R. BERNARD, Can. J. Zool. *29*, 80 (1951).
[11] L. HUOT and W. CORRIVEAULT, Arch. int. Physiol. Biochim. *68*, 577 (1960).

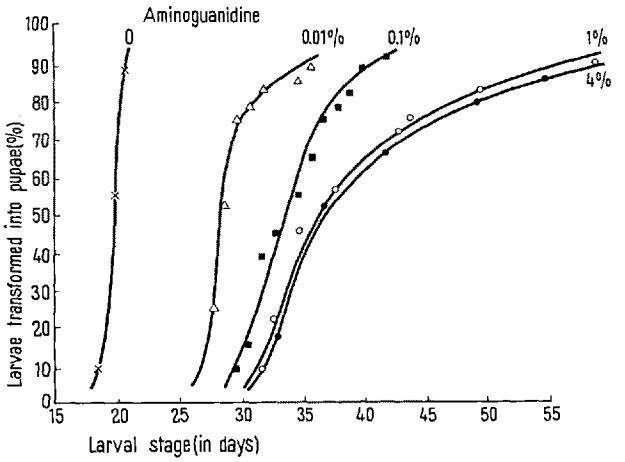
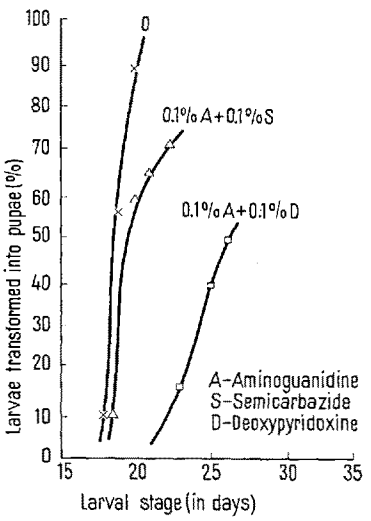
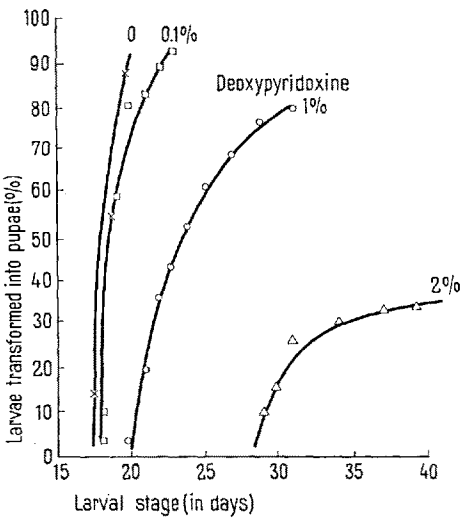


Fig. 1. Effect of aminoguanidine on larval stage of *Tribolium confusum*.





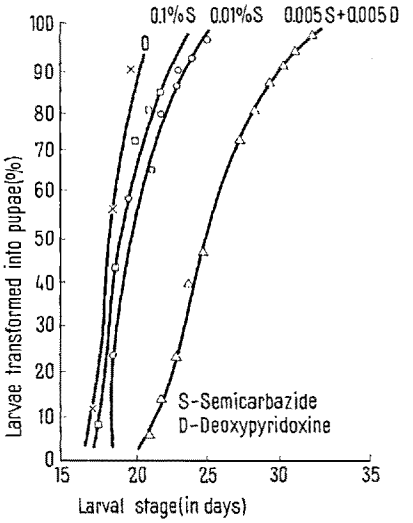


Fig. 2. Effect of semicarbazide used alone or with deoxypyridoxine on larval stage of *Tribolium confusum*. Fig. 3. Effect of deoxypyridoxine on larval stage of *Tribolium confusum*. Fig. 4. Effect of aminoguanidine combined with semicarbazide or with deoxypyridoxine on larval stage of *Tribolium confusum*.

Experiment 2: *Effect of semicarbazide on larval development.* This substance is known to inhibit a variety of enzymes of which decarboxylase and also histaminase. It is possibly for this reason that the rate of excretion of histamine in mammals is not greatly modified. In other words both the rate of histamine formation and destruction would be lowered so that the available histamine would remain relatively unchanged. In Figure 2 we see that semicarbazide has very little effect on growth. However at higher doses, i.e. at 1%, it was found to be 100% toxic to larvae.

Experiment 3: *Effect of deoxypyridoxine on larval growth.* This substance is known to be potent antimetabolite of pyridoxine. Its presence in a diet would inhibit all decarboxylase including the one for histidine and would therefore interfere with the formation of amines such as histamine, serotonine, etc. Figure 3 shows that deoxypyridoxine has some effect on larval growth but that this effect is not independent of a simultaneous toxic action contrary to aminoguanidine. Indeed at 2%, deoxypyridoxine retards growth but at the same time it is lethal to about 66% of the larvae as shown in Figure 3.

Experiment 4: *Effect of aminoguanidine combined with semicarbazide or deoxypyridoxine on larval growth.* The purpose of this study was to find out if the effect of aminoguanidine on larval growth, which is presumably due to histamine accumulation, could be prevented by inhibiting simultaneously the transformation of histidine into histamine. Figure 4 shows that semicarbazide almost completely prevents the effect of aminoguanidine on pupation and deoxypyridoxine exerts a similar action. It should also be mentioned that both these substances are slightly more toxic when used in association with aminoguanidine than when used alone as may be seen by comparing Figure 3 and 4.

Experiment 5: *Effect of semicarbazide combined with deoxypyridoxine on larval growth.* As shown in Figure 2 semicarbazide and deoxypyridoxine potentiate one-another and retard pupation at doses where used separately these substances are relatively inactive. This result in the light of the above mentioned experiments would seem to indicate that interference with amine formation results in inhibition of larval growth.

Discussion. In mammals histamine has marked effect on smooth muscles and exerts strong actions on the cardiovascular system. GILMOUR believes that this amine cannot have much effect on insects[12]. However since both histidine, the immediate precursor of histamine, and pyridoxine, the coenzyme of decarboxylase, are essential in *Tribolium confusum* and since as postulated by KAHLSON histamine could be involved in all growth processes, its importance in larval growth was investigated. Two methods of approach were used in this study which consisted first in interfering with histamine destruction by using aminoguanidine and in the second case in preventing histamine formation by decarboxylase inhibitors such as deoxypyridoxine or semicarbazide. Our results show that aminoguanidine, possibly by inhibiting histaminase and consequently increasing histamine concentration[13], has a marked effect on larval growth. This effect is non toxic since even at quantities 400 times higher than the minimum required effective dose for slowing of larval growth, the larvae are all transformed eventually into pupae. It seems then that excess histamine retards larval growth. When attemps were made to inhibit histamine formation it was found that this procedure did not affect growth significantly without becoming toxic. KAHLSON found that interference with histamine formation in mammals prevents fetus development[8]. Our study shows the same phenomenon to be true in insects. It would be interesting to see if excess histamine would retard growth in mammals as it does in insects.

It was shown recently that reserpine inhibits histaminase activity to the same extend than aminoguanidine[14]. On the other hand two of us have shown recently that reserpine inhibits larval growth[11]. When the effective concentration of reserpine and aminoguanidine needed for retardation of larval growth are compared it is found that both substances are active at the same concentration, i.e. approxymately 0,01% in diet. It is then possible that both reserpine and aminoguanidine retard larval growth because they increase histamine concentrations.

Résumé. Le rôle possible de l'histamine au cours de la croissance larvaire a été étudié. L'inhibition de l'oxydase des diamines au moyen de l'aminoguanidine, a fortement ralenti la croissance larvaire du *Tribolium confusum*. Cette substance, cependant, ne s'est pas avérée toxique. D'autre part l'inhibition des décarboxylases par le semicarbazide ou le deoxypyridoxine ne modifie pas la croissance sans devenir toxique.

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Department of Physiology, School of Medicine, and Department of Biology, School of Sciences, Laval University, Quebec City (Canada), September 25, 1961.

[12] D. GILMOUR, *The Biochemistry of Insects* (Academic Press, New York 1961).

[13] O. ARUNLAKSHANA, J. L. MONGAR, and H. O. SCHILD, J. Physiol. *123*, 32 (1954).

[14] K. S. SACHDEW, R. AIMAN, and M. V. RAJAPURKAR, Brit. J. Pharm. *16*, 146 (1961).

Adrenergic Blocking Action of Cysteamine

It had been found previously that cysteamine protects rats subjected to extreme hypoxia (barometric pressure 141 mm Hg, pO_2 = 30 mm Hg) and prolongs the survival time (unpublished data). In the present experiments, evidence is presented that cysteamine has an adrenergic blocking action and causes a rise in blood pressure of the rat by itself.

Rats of both sexes (170 to 210 g) were used and anaesthetized with urethane. The blood pressure was recorded through a cannula which was inserted into the carotid artery and connected with a mercury manometer. A small polythene cannula, 0.5 mm in diameter, was inserted into the jugular vein and was used for injecting drugs. In another group of animals, cysteamine was injected intravenously in a dose of 120 mg/kg, and 1 h later these animals were sacrificed. The adrenals were taken out and the amount of catecholamines in the adrenals was estimated biologically by rat blood pressure. The action of cysteamine on the hyperglycemic effect of adrenaline was studied in rabbits. For producing hyperglycemia, adren-