tissues in norepinephrine increased the intensity of the fluorescence but did not increase the amount of fluorescent nerve.

This suggests that the formaldehyde-induced fluorescence of catecholamines had detected most of the developing adrenergic nerves, a similar suggestion was made after administration of noradrenaline or inhibition of the metabolism[2,3].

The development of adrenergic nerves of both organs proceeded almost identically; however, the vas deferens seemed to develop its adrenergic innervation somewhat earlier than the ureter. Difference between both organs was also quantitatively significant: likewise in adult animals, the density of adrenergic nerve terminals is much lower in the ureter compared to the rich sympathetic innervation of the vas deferens[12].

The histochemical study demonstrated that before reaching the terminal part of the adrenergic nervous network, the outgrowing non-varicose nerve fibres had a larger diameter and a higher fluorescence intensity than the adult preterminal axons. The fluorescence of these non-terminal fibres decreased concomitantly with a progressive increase in the fluorescent varicosities of the developing terminal nerve fibres. Similar observations were made in other organs and species[3,4]. This suggests that during early development the adrenergic terminal nerve fibres move into, rather than form within, the effector organ to form the autonomic ground plexus.

At birth, the kidney is not fully differentiated[13,14], and it has been shown recently that full differentiation of the ureter is also not achieved at birth[15,16]. During development there appears to be a correlation between the amount of muscle and the presence of function. LEESON and LEESON[15] have observed that the rat ureter is composed of mature smooth muscle cells only by the 5th post-natal day and a fully developed lamina propria and muscularis are acquired over a period of 7 days. The rapid acquisition of a fully developed ureteral musculature coincides with the loss of the placenta as the principal excretory organ[15]. The fact that the ureter is devoid of functional adrenergic nerves at birth correlates well with the above-mentioned observations. The subsequent development of the adrenergic terminal innervation appears to be related to the underlying maturation of the smooth musculature.

Résumé. A la naissance, la musculature de l'uretère et du canal déférent du lapin est dépourvue d'une innervation adrénergique fonctionnelle. Les fibres nerveuses terminales apparaissent vers le troisième et quatrième jour et l'innervation augmente rapidement pour atteindre l'état adulte entre la quatrième et la sixième semaine. Le développement de l'innervation adrénergique périphérique est lié à la maturation sous-jacente de la musculature lisse.

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Stimulation of Cell Aggregation by Theophylline in the Asexual Reproduction of Fresh-Water Sponges (*Ephydatia fluviatilis*)

The gemmules of fresh-water sponges arise through the local aggregation in the mesohyle of the sponge of several types of amoeboïd cells. The first to aggregate are the archaeocytes, which will eventually become the vitellus-stuffed embryonic cells and the trophocytes at the expense of which the vitellus is build up by phagocytosis[1]. We have previously studied the physiology of gemmulation on populations of sponges of specified strain, age and size, grown in Petri dishes under various experimental conditions[2–5]. It has also been possible to observe the formation of a gemmule in very thin sponges[6], grown between 2 glass slides[7].

These experiments and observations strongly suggest that 1. the aggregation of cells during the building of a gemmule is oriented by a chemical signal and that 2. several of the physiological variables that modulate the frequency of gemmulation in a sponge population bear a striking resemblance to those implied in the aggregation of cellular slime-molds[8]. The identification of acrasin as cyclic 3′, 5′ adenosine monophosphate and of acrasinase as a phosphodiesterase[9–14] suggested that we should investigate a possible effect of either cyclic AMP or an inhibitor of phosphodiesterase on gemmulation.

Material and methods. We used fresh-water sponges of the species *Ephydatia fluviatilis*; strains α, β, γ and δ[15]. The sponges were cultivated from gemmules that had been gathered from open-air cultures in a pond near Brussels. Culture methods have been described in detail previously[2–4]. Briefly, the gemmules are incubated in a mineral medium, at 20 °C in the dark, except for some experiments bearing on the influence of light.

By grouping these gemmules in clusters before they hatch, we can control the individual size of the sponges as

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well as their overall population density in the dish. The ages of the sponges are counted in days elapsed from the start of incubation. The time it takes the sponges to hatch (generally 3 to 4 days) is therefore included in their age.

Results. 1. With theophylline: dose-response curve. Preliminary experiments had shown us that theophylline (pure, Knoll) enhances gemmulation at concentrations in the range of 10^{-4} M/l and is inactive at concentrations less than 10^{-5} M/l. Therefore, we restricted our dose-response experiments to concentrations in that range, with the purpose of determining the most convenient dose for later use.

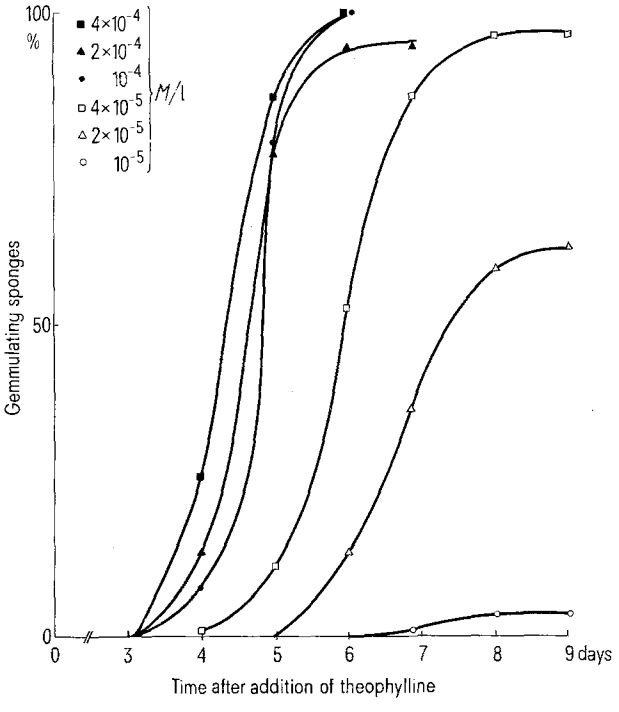

Fig. 1. Percentages of sponges that undergo gemmulation as a function of the duration of treatment with various concentrations of theophylline. The gemmulation rate of untreated controls is zero.

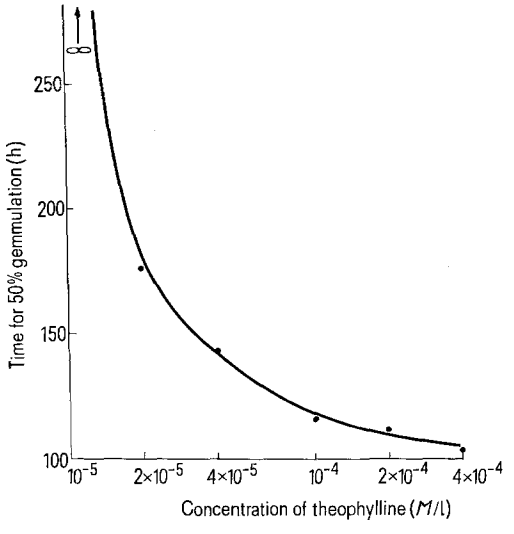

Fig. 2. Duration of exposure to various concentrations of theophylline needed by a sponge population to achieve a 50% gemmulation.

The experiments were made on populations of 30 sponges, in 25 ml Petri dishes, each sponge being hatched from 4 gemmules. Theophylline was added, to the proper concentrations, on day 7. Three Petri dishes were observed for each concentration.

The sponges were counted as gemmulating as soon as the local aggregation of archaeocytes was clearly visible, as a dense spot, under the stereomicroscope. In the case (that is frequent under conditions of strong stimulation) where 2 gemmules were being produced at the same time, the case was recorded as a single event. Figure 1 shows the gemmulation percentages as a function of age, Figure 2 shows the time at which a 50% gemmulation is achieved, as a function of concentration.

It can be seen from these data that 10^{-4} M/l is the lowest concentration at which a maximum speed of gemmulation is achieved and also the smallest one for which a 100% yield is attained in 1 week. The latter consideration makes stimulation by 10^{-4} M theophylline the more valuable for laboratory work that during the same period, the spontaneous gemmulation rate of untreated controls is nil.

Interaction of theophylline stimulation with other experimental conditions.

We have described preciously[4,5] the incidence on the gemmulation of sponges of some variables such as individual size, nutritional state, renewal of the medium, lighting conditions and strain-to-strain differences. A whole array of experiments has been made to assess the effect of theophylline in combination with these various factors. As an example, we may mention an experiment in which each experimental lot is made of 3 Petri dishes, containing 10 ml medium and 12 sponges, each of them being hatched from 4 gemmules. From day 8 on, the medium was renewed 3 times a week. Some cultures were kept in constant darkness (D), some others in a 25 lux light from incandescant bulbs (L). At each renewal of the medium, some cultures (N) were fed dead *E. coli* (8 µg nitrogen/ml culture), or given theophylline (T) (final concentration : 10^{-4} M/l). The Table gives the experimental results, expressed as numbers of gemmules, formed at day 21, per 100 sponges, in each experimental condition.

Without entering into repetitious details, we can sum up the results of this experiment and many others by saying that in every experimental condition we tried the gemmulation rate was significantly higher in the cultures treated with theophylline as in the untreated controls.

2. With cyclic nucleotides. Single applications of cyclic AMP (cryst. Boehringer), in concentrations ranging from 10^{-9} to 10^{-3} M/l, as well as of N^6-2′-*O*-dibutyryl 3′ 5′ adenosine monophosphate (monosodium, A grade, Calbiochem) and 3′, 5′ guanosine monophosphate (potassium

	α	β	γ	δ
D	0	0	0	0
D + T	100	100	0	83
D + N	11	3	0	3
D + N + T	140	136	77	170
L	0	0	0	0
L + T	31	0	0	12
L + N	0	0	0	0
L + N + T	104	72	67	43

Number of gemmules formed at day 21, per 100 sponges of different strains $\alpha, \beta, \gamma, \delta$ and in various experimental conditions. D, darkness; L, light; N, nourishment; T, theophylline. For values of these variables, see text.

dihydrate, A grade, Calbiochem) in concentrations ranging from 10^{-9} to 10^{-5} M/l, on unfed sponges, either at day 7 or at day 14, had no significant effect on gemmulation. Various combinations of repetitive addition of cAMP were also tried, without any positive result. A single application of 10^{-3} M cAMP alters reversibly the organisation of the sponge; 3 successive applications at 2 days intervals lead to an irreversible disorganization of the aquiferous system.

To sum up this aspect of our experiments, we can say that we did not encounter one experimental situation in which a rise in overall concentration of cAMP had any effect on gemmulation.

Discussion. It has been abundantly substantiated that, in vertebrates, theophylline and, more generally, methylxanthines, interact with many hormonal phenomena by inhibiting the cAMP-phosphodiesterases that help to regulate the intra-cellular concentration of cAMP[16]. In other organisms, however, the situation may be different: theophylline does not inhibit the cAMP-phosphodiesterase of *Escherichia coli*[17] and inhibits only slightly one of the extra-cellular phosphodiesterases of *Dictyostelium discoideum*[18].

In the present state of our knowledge of the biochemistry of sponges, it is very difficult to interpret our finding that theophylline stimulates gemmulation whereas a general application of cAMP does not. The two facts, however, are not in contradiction to the hypothesis that theophylline acts on gemmulation by enhancing a cAMP-dependant mechanism.

Cyclic AMP might be implied in gemmulation as a first messenger[19], acting in the aggregation of sponge cells in the same way as in the aggregation of some cellular slime-molds. In such a hypothesis, the signal for the coming together of amoeboïd cells would be a local concentration gradient of cAMP. Although such a gradient could not be mimicked by a rise in overall concentration of cAMP, its building up might be accelerated by the inhibition of cAMP phosphodiesterases in or around cAMP emiting cells.

In this respect, it is worth noticing that, on many occasions, theophylline stimulated sponges start producing at the same moment, in 2 different places, 2 gemmules of equal size, though smaller than a normal gemmule. In contrast, control sponges of the same size, when they gemmulate, always produce only 1 gemmule at a time. This might be an indication that theophylline acts on gemmulation by eliciting a local process, rather than by modifying the physiological state of the whole sponge.

However, cyclic AMP might even well be involved at the intracellular level, some triggering event of the gemmulation depending on the accumulation of cAMP in a particular cell-type. In this case, our contradictory results with cAMP and theophylline might be explained by differences in the permeability of the membrane of that cell-type to both agents.

Finally, it is possible that theophylline acts on sponges in a way that has nothing to do with the metabolism of cAMP. It is therefore essential that we acquire some knowledge about the existence and the properties of cAMP phosphodiesterases in these organisms, and about a possible secretion of cAMP during gemmulation. This problem is at present being dealt with in our laboratory.

Résumé. La formation de gemmules dans les éponges d'eau douce est fortement stimulée par la théophylline 10^{-4} M, mais non par une augmentation globale de la concentration en adénosine monophosphate cyclique dans le milieu.

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Lysosomal Function of Juxtaglomerular Granules

The juxtaglomerular apparatus or complex is made up of the macula densa, the lacis cells and the juxtaglomerular (JGC) or epithelioid cells of the afferent glomerular arteriole. The macula densa, a portion of the distal tubule, is in intimate contact with the vascular pole of the glomerulus. The lacis cells of OBERLING and HATT[1] are located between the afferent and efferent arterioles and the macula densa[2]. The juxtaglomerular granules (JGG) of the JGC are currently recognized as the site of renin synthesis and storage[3–8].

Microdissection studies have verified that renin activity is localized in the afferent arteriole[7]. A good correlation has been established between the renal pressor activity and the number of granules in JGC[8]. The pressor activity has been shown to be localized in JGG[9], in which renin has been identified by immunofluorescence[10]. Electron microscopic studies on the JGC of rats with unilateral renal ischemia[11], magnesium[12,13] or sodium deficiency[14] have revealed numerous, variably electrondense, homogeneous JGG enclosed by a unit membrane.

Our investigation into the possible lysosomal function[15] of JGG was prompted by their ultrastructure as well as by the presence of renin (a proteolytic enzyme) and acid

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