On the other hand, doses of 10, 30, and 100 μg of TP diminished the growth produced by 0·3 μg of oestrone[1]. Thus a ratio of 3:1 to 10:1 testosterone to oestriol was inhibitory whereas a ratio of at least 33:1 testosterone to oestrone was required to produce inhibition. Testosterone would appear to be at least three times more effective as an oestriol antagonist than as an oestrone antagonist.

Thus the uterine growth produced by oestriol at a single dose of 100 μg was blocked by the simultaneous administration of testosterone propionate. On the other hand, this growth was not significantly affected by cortisone, cortisol, desoxycorticosterone, or progesterone. Oestrone-induced uterine growth (0·3 μg) in a test of this type was inhibited by DCA and aldosterone, testosterone propionate, and progesterone – only the glucocorticoids were inactive among naturally occurring, non-oestrogenic types of steroids. This difference in pattern of blockage led us to suggest that there were two receptor sites for steroids in the uterus[5]. According to this hypothesis one site (A) would accept oestrone, DCA, progesterone, and testosterone propionate and the other site (B) oestriol and testosterone. Further experiments (unpublished) have forced a modification of this hypothesis since some of the oestriol appears to attach to site A. Additional studies are in progress in an attempt to complete this dual receptor site hypothesis and to clarify the mechanisms involved in uterine growth.

R. A. Edgren and D. W. Calhoun

Division of Biological Research, G. D. Searle & Co., Chicago (Illinois), October 1, 1959.

Zusammenfassung

Das in juvenilen Mäusen durch Oestriol (100 μg) stimulierte Uteruswachstum wurde durch Testosteronpropionat blockiert. Mit Cortisonacetat, Cortisolacetat, Deoxycorticosteronacetat und Progesteron wurde keine solche Wirkung erzielt.

[5] R. A. Edgren and D. W. Calhoun, Anat. Rec. *134*, 558 (1959).

Stimulation of Plant Development by Some Uracil Analogues

Some time ago we were able to show that the organs of germinating seeds are the site of intensive nucleic acid synthesis[1]. For this reason, the first stages of germination appear to be particularly suitable for studying the effects of biologically active analogues of nucleic acid components. In pursuance of this line of investigation, we had earlier studied[2] the effect of 5-bromouracil, a compound known to inhibit the growth of certain microorganisms[3,4], on germinating plants, and found that at certain concentrations it not only failed to inhibit growth, but markedly stimulated development of the plants. A number of further derivatives of uracil were examined for analogous effects, and a number of them were found to stimulate the development of certain plants not only during germination, but also during the later vegetative period[5].

In the last few years, we have carried out field trials on crop plants using 5-bromouracil and 5-nitrouracil, which had proved most effective in laboratory experiments. The seeds were treated as in earlier work, by soaking them in solutions containing 5 to 50 μg of the stimulators per ml for 24 h just prior to sowing. The most general result of such a single treatment with 5-bromouracil is an accelerated development of the plants which in some cases may persist up to the time of harvesting. For instance, treated tomatoes on the average ripen 10 to 14 days earlier than untreated controls, and in the case of lettuce the first harvest from treated seeds is about 20% higher than from controls. The effect of 5-nitrouracil on lettuce is similar. The same compound increases the yield of cucumbers to a statistically significant extent – about 20–40% above that of controls (seeds soaked in water for the same period). The cucumbers treated with 5-nitrouracil show certain morphological peculiarities in the course of development. The ovaries of such plants begin to elongate much earlier so that at the full development of the perianth they are 4–5 times longer than the ovaries of control plants at the same stage of development. The ovaries of the treated plants do not lie along the ground, as is usual with cucumbers but are characteristically erect.

In an effort to elucidate the mechanism of stimulation, we first studied the tissues of stimulated plants cytologically. In certain cases, the stimulators were found to cause a massive increase in the number of mitoses. Thus, in the roots of onions (*Allium cepa*) allowed to sprout in aqueous solutions of the stimulators, the number of mitoses after 4 to 8 days is markedly higher than in the controls sprouting in water; 5-bromouracil at 10 μg/ml and 100 μg/ml was found to increase the number of mitoses by 30% and 60% respectively on the fourth day, and by 70% and 35% respectively on the eighth day.

We further attempted to follow the metabolic fate of 5-bromouracil in plants. The experiments were carried out with germinating cucumbers grown under standard conditions and infiltrated with 0·001 *M* solutions of 5-bromouracil-2-^{14}C[6]. After 3 h and 18 h, no combined 5-bromouracil was found in the nucleotide fraction, nor in the ribonucleic or deoxyribonucleic acids obtained according to Ogur and Rosen[7]. This finding indicates that the fate of 5-bromouracil in the plant organism differs distinctly from its fate in microorganisms in which it is known to be able to replace a considerable proportion of the thymine in deoxyribonucleic acid[8]. This difference is presumably due to the fact that 5-bromouracil is rapidly degraded in the plant. When seedlings are incubated after infiltration with 5-bromouracil, this degradation may be followed by the evolution of $^{14}CO_2$, which reaches a maximum after only 1 h incubation. However, 5-bromouracil does have a distinct effect on the metabolism of the purine and pyrimidine bases, particularly of uracil. Plants infiltrated with uracil-2-^{14}C together with (unlabelled) 5-bromouracil show a distinctly decreased rate of degradation of uracil to $^{14}CO_2$ as against control plants infiltrated

[1] Z. Šormová and F. Šorm, Chem. listy *50*, 629 (1956); Coll. Czechosl. chem. Comm. *21*, 1043 (1956).

[2] Z. Šormová, F. Šorm, J. Bauerová, and M. Zelinková, Fiziologia rastenij *3*, 204 (1956).

[3] G. H. Hitchings, E. A. Falco, and M. B. Sherwood, Science *102*, 251 (1945).

[4] F. Weygand, A. Wacker, and H. Dellweg, Z. Naturf. *7b*, 19 (1952).

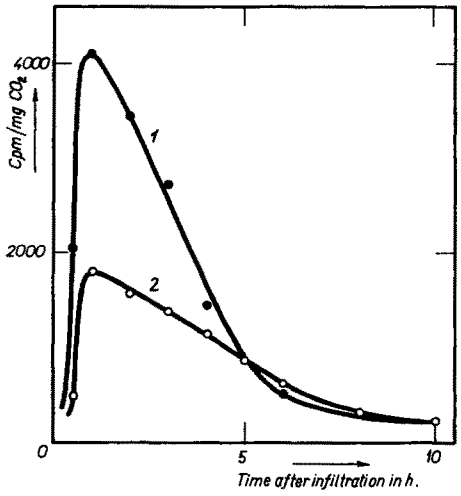
[5] Czechoslovak Patent 87672.

[6] The labeled 5-bromouracil was prepared by Dr. J. Morávek of the Institute for Research, Production, and Utilisation of Radioisotopes, Prague.

[7] M. Ogur and G. Rosen, Arch. Biochem. *25*, 262 (1950).

[8] S. Zamenhof, B. Reiner, R. de Giovanni, and K. Rich, J. biol. Chem. *219*, 165 (1956).

with the labelled uracil alone (Fig.). At the same time, there is an increase in the specific activity of the free uracil nucleotides; even after 20 h incubation, the specific activity of these nucleotides is still twice as high as in the control group. If labelled orotic acid is substituted for uracil in an analogous experiment, there is no corresponding increase in the specific activity of the free nucleotide fraction in the presence of unlabelled 5-bromouracil. The infiltration of labelled uracil together with unlabelled 5-bromouracil was also found to lead to a distinct increase in the specific activity of the ribonucleic and deoxyribonucleic acid fractions after 20 h – by 40% and 125% respectively above the values of the control group.



Inhibition of the degradation of uracil by 5-bromouracil. The specific activity of the CO_2 was determined after absorption in 0·25 *N* NaOH and precipitation of $BaCO_3$. The values are corrected for self-absorption. The total amount of CO_2 evolved was the same in both experiments. 1: after infiltration with 0·3 *mM* uracil-^{14}C; 2: after infiltration with 0·3 *mM* uracil-^{14}C 1·0 *mM* 5-bromouracil. The activities were measured in the Isotope Laboratory of this Institute

Our results show that these uracil derivatives exert profound effects on the development and metabolism of plants. The protracted nature of these effects is all the more interesting in view of the fact that they result from only a single treatment with low concentrations of the active substances in the earliest stages of germination. This, together with the observed morphological changes, points to the possibility of genetic effects; this problem is at present under investigation.

This work will be reported in full in the Collection of Czechoslovak Chemical Communications.

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Department of Biochemistry, Institute of Chemistry, Czechoslovak Academy of Science, Prague, December 4, 1959.

Zusammenfassung

Es wurde eine entwicklungsfördernde Wirkung von 5-Bromuracil und 5-Nitrouracil bereits in geringen Mengen auf manche Pflanzen (unter anderem Tomaten, Gurken und Gartenlattich) festgestellt. 5-Bromuracil wird dabei nicht in die Pflanzennukleinsäuren eingebaut, sondern unterliegt einem schnellen Abbau; weiterhin verursacht der Stoff eine Hemmung des Uracilabbaues. Die Möglichkeit einer Beeinflussung der genetischen Eigenschaften wird diskutiert.

Cytologie fine des cellules interstitielles du testicule du poisson *Lebistes reticulatus* R.

Les travaux de CHAMPY[1], VAN OORDT[2], STEPHAN et CLAVERT[3] ont posé le problème de l'existence et de la morphologie de ces cellules dans le testicule des Cyprinodontes. Chez *Lebistes reticulatus* R.[3] les cellules interstitielles actives sont caractérisées par la présence de granules éosinophiles et d'inclusions lipidiques soudanophiles.

Résultats. Les techniques histologiques et histochimiques permettent de distinguer 3 catégories de cellules interstitielles:

1° des cellules à cytoplasme clair dont le noyau volumineux se colore d'une façon homogène et intense à l'hématoxyline ferrique (ces éléments prédominent dans le testicule jeune);

2° des cellules à cytoplasme chargé en granulations éosinophiles;

3° des cellules contenant des mottes grossières de pigment jaune.

Le nombre des cellules à pigment augmente sensiblement avec l'âge du poisson. Les pigments accumulés résistent aux solvants des graisses. Sur coupes à la paraffine colorées à l'hématoxyline éosine, ils présentent leur couleur propre jaune orangé. Par ailleurs, ils présentent les caractères généraux de coloration des lipides: osmiophilie, coloration au noir soudan et au bleu de nil. Ils se colorent assez fortement au P.A.S. Le test de Schmorl est fortement positif mais la réaction argentaffine selon Masson reste négative. L'ensemble de ces caractéristiques histochimiques permet de conclure à des pigments de nature lipidique, de la famille des lipofuscines.

Au microscope électronique (fixation Palade; méthacrylate de butyle – Philips E. M. 100), les contours des cellules interstitielles sont très irréguliers; de nombreuses digitations sont plus ou moins intriquées. Souvent elles ne sont séparées des capillaires que par un mince espace clair contenant des fibrilles collagènes. Deux caractères différencient ces cellules d'éléments conjonctifs banaux:

a) leurs mitochondries volumineuses et denses renfermant de fins tubules d'un diamètre de 400 Å environ. Ce type de mitochondrie a été décrit dans les cellules interstitielles du testicule de mammifère (FAWCETT et BURGOS[4]) et d'une façon générale dans les organes producteurs de stéroïdes (BELT et PEASE[5]);

b) leur richesse en vésicules, souvent disposées en chaînettes, constituant le système réticulaire endoplasmique.

Les grains de Palade, peu nombreux, dispersés dans la matrice cytoplasmique, ne sont que rarement disposés sur la paroi externe des vésicules du système réticulaire endoplasmique: ces vésicules sont en majorité de type lisse. Le noyau arrondi de contour régulier, présente un aspect normal et renferme une substance nucléaire finement granuleuse, répartie de façon homogène. Le nucléole

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