Action of Puromycin and 5-Fluorouracil on the Adrenal Responsiveness to Corticotrophin

The concept of the messenger RNA in molecular biology and the possibility of inducing and repressing enzymes in bacteria has given new impetus to the search for a mechanism of hormone action. One of the questions which seems to be of special interest is whether hormones which initiate certain metabolic processes act by activating pre-existing enzymes or whether they cause the actual net synthesis of new enzymes. The effect of adrenocorticotropin (ACTH) on the output of corticosteroids by the adrenal lends itself especially well to the study of this question since much about the hormone action is known. Thus it was shown by HAYNES and BERTHET[1] that adrenal α-glucan phosphorylase is increased upon incubation with ACTH. The finding that cyclic 3′, 5′-AMP is causing the transformation of phosphorylase b to phosphorylase a via the activation of phosphorylase kinase[2] as well as the fact that steroid output can be increased by TPN and glucose-6-phosphate[3] has led to the proposition of the following chain of events:

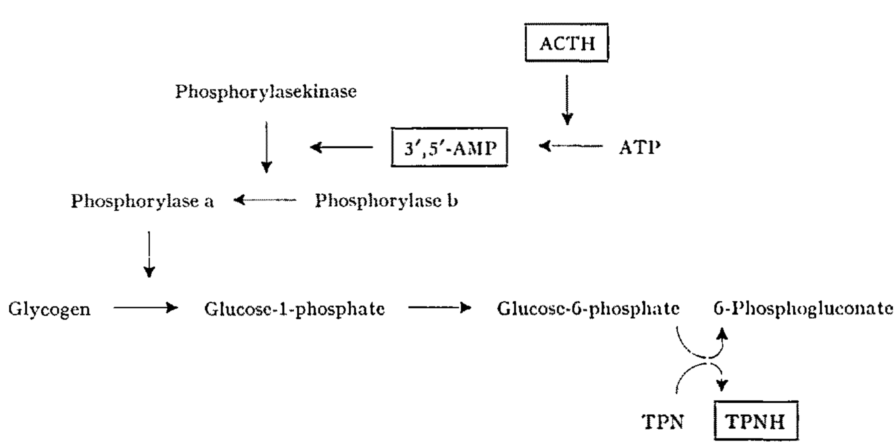
The experiments to be reported were performed to determine the effect of the three agents ACTH, cyclic 3′, 5′-AMP and TPN on corticosteroid production under the influence of protein synthesis inhibitors, and thus to determine whether new synthesis of enzymes was required for ACTH-action.

Methods. Quartered adrenal glands from rats were incubated in Krebs-Ringer bicarbonate pH 7.4 (total volume 1.5 ml) according to SAFFRAN and SCHALLY[4]. Various agents such as 50 mU ACTH, 3 mg 3′, 5′-cyclic AMP or 1 mg each of triphosphopyridine nucleotide (TPN, NADP) and glucose-6-phosphate were added in the presence or absence of 1 mg puromycin. In the experiments in which RNA synthesis was inhibited by 5-fluorouracil the rats were pretreated with 150 mg/kg of fluorouracil[5] intraperitoneally 2 h before sacrifice. Corticosteroids were measured in the medium by extraction with methylene chloride followed by the blue tetrazolium method of ELLIOTT et al[6].

Results. 5-Fluorouracil, a pyrimidine analogue, inhibits protein synthesis by interfering with the synthesis of RNA[7]. Its effect upon protein synthesis in our experiments was demonstrated by the finding that ^{14}C-leucine incorporation into diaphragms of the same rats from which the adrenals were used was inhibited by about 40%. As shown in Table I the effect of ACTH and 3′, 5′-cyclic AMP is greatly diminished in rats treated with 5-fluorouracil as compared to untreated animals. TPN and glucose-6-phosphate, in contrast, show similar effects in treated and untreated animals.

An effect similar to that observed under the action of 5-fluorouracil but in the presence of puromycin has been reported by FERGUSON[8]. Puromycin, added in vitro, inhibits the action of ACTH and cyclic 3′, 5′-AMP, whereas the addition of TPN plus glucose was still capable of causing an increase in steroid output comparable to that of untreated adrenals. As shown in Table II, we have been able to confirm these results.

Both agents, 5-fluorouracil and puromycin, therefore, seem to act in a similar way, i.e. by preventing the action of ACTH and cyclic 3′, 5′-AMP without affecting the action of TPN plus glucose. The effect of cyclic 3′, 5′-AMP is of special interest, since this compound is known to act on the isolated phosphorylase system directly by activating phosphorylase kinase rather than by causing net enzyme synthesis. In the accompanying Figure three enzymes are located between the action of cyclic 3′, 5′-AMP and that of TPN and glucose, i.e. phosphorylase kinase, phosphorylase and phosphoglucomutase. An inhibition of the action of cyclic 3′, 5′-AMP without that of TPN would seem to indicate that any one of these three enzymes can no longer be stimulated. However, a similar effect would also occur if glycogen were not sufficiently available as a substrate. Puromycin has been reported to decrease the glycogen in various organs, especially in liver[9]. Similarly 5-fluorouracil is capable of interfering with the synthesis of UDPG, thus also affecting glycogen synthesis.



A quantitative determination of the available glycogen seemed rather difficult in view of the variable location of glycogen in different layers of the adrenal cortex reported

[1] R. C. HAYNES JR. and L. BERTHET, J. biol. Chem. *225*, 115 (1957).
[2] E. G. KREBS, D. J. GRAVES, and E. H. FISCHER, J. biol. Chem. *234*, 2867 (1959).
[3] S. B. KORITZ and F. G. PÉRON, J. biol. Chem. *230*, 343 (1958).
[4] M. SAFFRAN and A. V. SCHALLY, Endocrinol. *56*, 523 (1955).
[5] Kindly provided by F. Hoffmann-La Roche & Co. Limited, Basel.
[6] F. H. ELLIOTT, M. K. BIRMINGHAM, A. V. SCHALLY, and E. SCHÖNBAUM, Endocrinol. *55*, 721 (1954).
[7] L. BOSCH, E. HARBERS, and CH. HEIDELBERGER, Cancer Res. *18*, 335 (1958).
[8] J. J. FERGUSON, Biochim. biophys. Acta *57*, 616 (1962).
[9] J. HOFERT, J. GORSKI, J. C. MUELLER, and R. K. BOUTWELL, Arch. Biochem. Biophys. *97*, 134 (1962).

by GREENBERG and GLICK[10]. The interference with the glycolysis by puromycin could, however, be demontrated in the following way. As can be seen in Table II, steroid output by adrenal slices can also be stimulated by TPN alone in the absence of glucose-6-phosphate. Apparently the endogenous glycolysis provides enough glucose-6-phosphate for the TPN to act. But in the absence of exogenous glucose-6-phosphate there is a marked difference in the action of TPN in the presence or absence of puromycin. Puromycin, as well as inhibiting the action of ACTH and cyclic 3',5'-AMP, also inhibits the action of TPN alone in the absence of glucose-6-phosphate.

Table I. Corticosteroid production by adrenal slices of controls and pretreated animals after stimulation by ACTH, 3',5'-AMP, and TPNH

	Untreated	Treated with 5-fluorouracil
Controls	6.1	4.4
ACTH 50 mU	15.8	8.5
3',5'-AMP 3 mg	16.3	7.6
TPN + glucose-6-phosphate 2 mg + 3 mg	10.7	9.3

Table II. Stimulation of steroid production in adrenal slices by various agents in the presence and absence of puromycin

	— Puromycin	+ Puromycin
Controls	2.5	
ACTH	20.0	4.4
Controls	3.0	
Cyclic 3',5'-AMP	16.0	2.7
Controls	3.7	
TPN + glucose-6-phosphate	7.4	7.4
Controls	3.2	
TPN (no glucose-6-phosphate)	6.9	3.2

The fact that under the influence of 5-fluorouracil and puromycin the ACTH effect is reduced does not necessarily mean that synthesis of a new protein is required for ACTH action; it could also be due to a depletion of glycogen, in which case the activation of phosphorylase would no longer result in an increased energy supply.

Zusammenfassung. Puromycin und 5-Fluorouracil beeinträchtigen in Nebennierenschnitten der Ratte die durch ACTH und cyclisches 3',5'-Adenosinphosphat stimulierte Corticosteronausscheidung. Die durch TPN plus Glukose-6-phosphat stimulierte Corticosteronausscheidung bleibt unbeeinflusst. Dagegen bewirkt Puromycin bei Zusatz von TPN allein eine Hemmung. Die Wirkung von Puromycin und 5-Fluorouracil dürfte daher eher auf die Beeinflussung des Energiestoffwechsels als auf eine Hemmung der Neubildung induzierter Enzyme zurückzuführen sein.

M. STAEHELIN

Forschungslaboratorien der CIBA Aktiengesellschaft, Pharmazeutische Abteilung, Basel (Switzerland), January 22, 1965.

[10] L. J. GREENBERG and D. GLICK, J. biol. Chem. *237*, 3552 (1962)

The Increase of Frost Resistance of Tomato Plants through Application of 2-Chloroethyl Trimethylammonium Chloride (CCC)

CCC, which is closely related to choline, belongs to the group of plant-regulating substances that retard the growth of plants without injurious effects. This compound is also interesting because of its faculty to build up, in certain plants, a resistance against unfavourable environmental conditions, like dryness[1-4], excessive salt content[4-6] or unsuitable pH[7].

In this paper the effect of CCC on the germination of tomato seeds at unfavourable temperatures, and also on frost resistance of tomato seedlings, was investigated. The seeds were soaked in aqueous solutions of CCC (10, 100, and 500 ppm) or in water for 24 h at 10°C and then germinated in Petri dishes (100 seeds per dish) at the optimal (25°C) and minimal (12°C) temperature.

The positive effect of CCC on the germination was observed only at the low temperature (12°C) when a concentration of 500 ppm was applied (Table I). The experiments on plant resistance against low temperature were carried out under ordinary conditions during the winter of 1963/64. Control plants as well as those treated with

Table I. Number of germinated seeds in a temperature of 12°C shown in %

Days of germination	Concentration of CCC in ppm 0	10	100	500	L.S.D. at $P = 0.05$
6	9.6	10.5	10.2	14.0[a]	3.46
10	43.6	44.0	43.8	53.0[a]	8.39
15	65.0	63.8	63.5	68.6	5.63

[a] Significant differences in relation to control.

[1] R. S. LINDSTROM and N. E. TOLBERT, Mich. agr. exp. Sta. Quart. Bull. *42*, 917 (1960).
[2] H. A. HALEVY and B. KESSLER, Nature *197*, 310 (1963).
[3] M. MICHNIEWICZ and A. CHROMIŃSKI, II Sympoz. Growth Regul. Toruń (1963), Zeszyty Nauk U.M.K., in press.
[4] H. E. DAMATY, H. KÜHN, and H. LINSER, Agrochimica *8*, 129 (1964).
[5] P. C. MARTH and J. R. FRANK, J. agr. Food Chem. *9*, 359 (1961).
[6] T. MIYAMOTO, Naturwissenschaften *49*, 213 (1962).
[7] T. MIYAMOTO, Naturwissenschaften *49*, 377 (1962).