prior to mitosis, and the importance of the maintenance of a critical level of ATP in cleaving sea urchin eggs for continued occurrence of mitoses has been recently demonstrated[24]. Actinomycin, by inhibiting energy-utilizing macromolecular syntheses, might, at late interphase, induce prematurely a situation similar to that reported for premitotic *Tetrahymena*. A comparison of the energy metabolism in actinomycin-treated plasmodia with that of premitotic plasmodia should be of interest under this point of view[25].

Zusammenfassung. Actinomycin D (125 µg/ml) bewirkt prophaseartige Kernveränderungen in den Plasmodien des mitotisch synchronen Schleimpilzes *Physarum polycephalum* bei etwa zweistündiger Einwirkung, im Zeitraum von weniger als etwa 5 h vor Mitosebeginn.

E. GUTTES and SOPHIE GUTTES[26]

Department of Biology, Brown University, Providence (Rhode Island, USA), January 2, 1964.

[24] D. EPEL, J. Cell Biol. *17*, 315 (1963).

[25] We are indebted to Dr. C. A. STONE, Merck Institute for Therapeutic Research, West Point (Pennsylvania), for providing us with actinomycin D.

[26] Present address: *Department of Biology, Loyola University, Chicago (Illinois, USA).*

Adaptation of the Guinea-pig to Histamine. Sensitivity of the Guinea-pig's Ileum to Histamine and Acetylcholine[1]

The process of physiological adaptation of an organism develops on different functional levels including nervous and humoral elements. In consequence this process manifests itself by changes in responsiveness of the respective effectors.

Guinea-pigs may be adapted to histamine; it is an open question whether this process involves the bronchi only, on which this adaptation has been demonstrated[2]. We thought it interesting to see whether other smooth muscle structures were also involved, and we therefore studied the guinea-pig ileum.

Methods. Isolated pieces of ileum of 25 guinea-pigs, males and females, 300–450 g of body weight were used. Of these animals, 16 had been adapted to histamine (H) by daily intraperitoneal injections beginning with a dose of 0.24 mg/kg of histamine[3], which was raised every 5 days by 0.03 mg of H to the terminal adaptation dose of 0.42 mg/kg. This took 6 to 8 weeks. Prolongation of tolerance time of an animal in histamine aerosol[4] under conditions previously described[5] served as a criterion of histamine adaptation.

Isolated ilea of 9 non-adapted guinea-pigs were used as controls. Every isolated ileum was suspended in oxygenated Tyrodé's solution without atropine, according to Magnus's method as modified by MAŚLIŃSKI and CZEKALIŃSKI[6]. The temperature of the bath was maintained at 33°C. Histamine and acetylcholine added to the bath were in contact with the ileum for 10–15 sec before rinsing. Sensitivity of isolated pieces of ileum to the doses of 0.0012, 0.0024, 0.0048, 0.0096, and 0.0192 µg of histamine in 1 ml of the bath, and 0.005, 0.01, 0.02, 0.04, and 0.08 µg of acetylcholine chloride[7] in 1 ml of the bath, was tested. A photooptic method of registration was employed.

Histaminase activity in homogenated ileum samples was determined by the method described by KAPELLER-ADLER[8].

Results. The dose-response curves of the ileum of adapted and control guinea-pigs to H are presented in Figure 1. The responsiveness of the ileum to acetylcholine is presented in Figure 2. The histaminase activities were as follows: Ileum of 9 adapted guinea-pigs = 4.0, 5 = 0.98 PU/0.125 g fresh tissue[9]. Ileum of 5 non-adapted guinea-pigs = 4.1, = 5 1.1 PU/0.125 g fresh tissue.

Discussion. The data presented reveal a change in histamine reactivity of ileal smooth muscle from guinea-pigs adapted to H. The change in reactivity is expressed by a significant decrease of contraction height. The change of H reactivity of the guinea-pig ileum is not paralleled by a significant change in acetylcholine sensitivity, as shown in Figure 2.

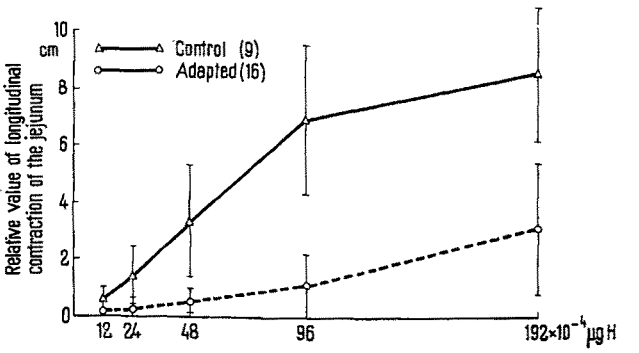

Fig. 1. The points on the curves represent mean values with standard deviations in cm of light beam deflection on administering histamine. The difference of mean values in two groups of the experiment on administering the dose of 0.0096 µg of histamine is statistically significant, $p = 0.001$. In parentheses number of guinea-pigs used in the experiment are shown.

[1] The study was supported by the Polish Academy of Sciences.

[2] Cz. MAŚLIŃSKI, S. M. MAŚLIŃSKI, and H. WEINRAUDER, Exper. *19*, 258 (1963).

[3] Histamine dihydrochloride Polfa, calculated as histamine base; further expressed quantities of histamine are calculated in the same manner.

[4] Histamine dihydrochlorid, Hoffmann-La Roche.

[5] Cz. MAŚLIŃSKI, J. Physiol. (P) *54*, 375 (1962).

[6] Cz. MAŚLIŃSKI and L. CZEKALIŃSKI, Post. Hig. Med. Dośw. *13*, 105 (1959).

[7] Acetylcholine chloride Polfa.

[8] R. KAPELLER-ADLER, Biochem. J. *44*, 70 (1949).

[9] PU = permanganate unit.

It is remarkable that despite the great difference of H reactivity between control and adapted animals there is no such difference in histaminase activities. This suggests that the enzymatic process does not play a basic role in the development of smooth muscle adaptation to histamine[10].

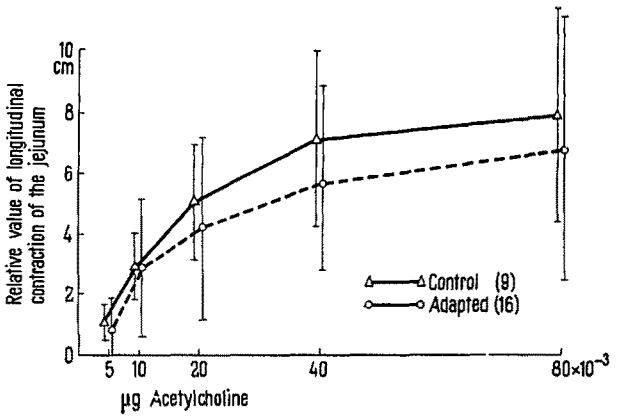

Fig. 2. The points on the curves represent mean value with the standard deviation of the ileum contraction in cm after administration of acetylcholine. The corresponding points on these two curves show no significant difference. In parentheses number of guinea-pigs used in the experiment are shown.

Résumé. L'adaptation des cobayes à l'histamine par voie intrapéritonéale diminue la sensibilité de leur jéjunum isolé à l'histamine. En même temps le jéjunum isolé de cobayes adaptés réagit à l'acétylcholine sans changer remarquablement de susceptibilité. L'activité de l'histaminase dans le jéjunum adapté ne démontre pas la différence en relation avec celle du jéjunum non adapté.

S. W. ANDRZEJEWSKI and ALWINA AUGUSTYNIAK

Department of General and Experimental Pathology, School of Medicine, Lódz (Poland), November 18, 1963.

[10] We thank Hoffmann-La Roche Ltd. for kindly supplying Histamine dihydrochlorid.

Dual Site of Action of Phenoxybenzamine in the Cat's Spleen: Blockade of α-Adrenergic Receptors and Inhibition of Re-uptake of Neurally Released Norepinephrine

Whether the sites primarily involved in the removal of exogenous and endogenous norepinephrine in a sympathetically innervated organ like the cat's spleen are the receptors of the effector organ, as proposed by BROWN and GILLESPIE[1,2] or rather sympathetic nerve endings, as advanced by PATON[3], is still a matter of controversy. We recently reported[4] that in the isolated perfused spleen of the cat, when removal of infused norepinephrine had been almost completely prevented by cocaine, blockade of smooth muscle receptors by phenoxybenzamine failed to increase further the amount of norepinephrine appearing in the venous effluent. This was taken as evidence that combination of norepinephrine with receptors of the effector organ was of only minor importance for the inactivation of exogenous norepinephrine. It might be objected that the mechanisms of inactivation are not identical for infused and neurally released norepinephrine. The results of the present study, however, lead us to believe that our previous conclusions may be applied to neurally released norepinephrine as well.

Isolation and perfusion of the spleen was performed as described in detail by THOENEN et al.[5]. The splenic nerves were stimulated at 8 min intervals for 10 sec at a rate of 6/sec, with monophasic rectangular pulses of 1 msec duration. Supramaximal voltage was used. Volume changes were recorded with a piston recorder and perfusion pressure with a Condon type Hg-manometer, the pressure changes being a measure of vascular resistance, since perfusion rate (7.5 ml/min) was kept constant. At the onset of every stimulation period the venous effluent was collected in chilled graduated centrifuge tubes for 90 sec. After this time the noradrenaline concentration had returned to levels below the sensitivity of the assay method in untreated preparations. Under phenoxybenzamine infusion the return to prestimulation levels was delayed and the collecting period was prolonged to 150 sec. The norepinephrine content of the venous effluent was assayed on the blood pressure of pithed rats.

After several stimulation periods with constant effect on volume and vascular resistance an infusion of 3 µg/min followed by 10 µg/min of phenoxybenzamine was started. The concentrations perfusing the spleen thus amounted to 0.4 and 1.3 µg/ml. The addition of corresponding amounts of phenoxybenzamine to norepinephrine test solutions had no influence on the norepinephrine assay.

The extent and time course of the adrenergic blocking effect and of the effect on the norepinephrine output during phenoxybenzamine infusion are shown in a representative experiment in Figure 1. In Figure 2 the results of 8 experiments are summarized. For better comparison relative rather than absolute terms were used, since in the control periods the changes in volume and vascular resistance as well as the norepinephrine output varied considerably from one experiment to another, whereas these values were remarkably constant in the same preparation. The contraction area (area enclosed by the lever's writing point from the beginning of the stimulation to its return to the starting level) was chosen as a representative measure of the mechanical response including

[1] G. L. BROWN and J. S. GILLESPIE, J. Physiol. *138*, 81 (1957).
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[3] W. D. M. PATON, *Adrenergic Mechanisms*. Ciba Foundation Symposium (J. A. Churchill, London 1960), p. 124.
[4] H. THOENEN, A. HÜRLIMANN, and W. HAEFELY, Exper. *19*, 601 (1963).
[5] H. THOENEN, A. HÜRLIMANN, and W. HAEFELY, Helv. physiol. pharmacol. Acta *21*, 17 (1963).