THE INHIBITORY EFFECT OF PUROMYCIN ON ADAPTATION OF GUINEA-PIG TO AEROSOLS OF ACETYLCHOLINE AND HISTAMINE

JUSTYNA M. WISNIEWSKA* AND J. S. KNYPL**

DEPENDING on a season male guinea-pig can be adapted to 1·5% acetylcholine (ACh) ærosol[1] within a week in September-October or within 2-3 weeks in summer.[2] Since adaptation to ACh is about three times stimulated by cysteine[3] and in blood of the adapted animals activity of acetylcholine acetylhydrolase (AChH) increases to 130–150% of the control value,[2] it was necessary to test whether the process of adaptation will be affected by puromycin (PMC), known as a specific inhibitor of protein synthesis[4] and the inhibitor of AChH synthesis in chick embryo tissues.[5]

Increment of tolerance to ACh during the adaptation is coupled with the parallel increment of tolerance to histamine (H) ærosols, and *vice versa*.[2] For that reason the effect of PMC on adaptation to H was also studied.

Experiments were carried out on coloured male guinea-pigs weighing 260–290 g. PMC was injected intraperitoneally 3-4 hours before the exposition either in aerosol of ACh or H. Adaptation to ACh was carried out as described previously.[6] The animals once a day were exposed in the aerosol produced by generator D-30 modified in this laboratory as a model D-30 *a*.[7] A period of time, in sec., passing between a moment of introduction of the animal into the expositional chamber to a moment of appearance of initial symptoms of the third phase of induced asthmatic-like attack,[8] *i.e.*, bradypnoæ, was taken as a measure of the actual ACh-tolerance. Guinea-pig which survived in the atmosphere of aerosol for 20 min. was regarded as the adapted one.[6] Adaptation to 0·5% aerosol of H was carried out in the same manner. AChH activity in blood samples of the ACh-adapted animals was determined according to Hestrin's colorimetric method as modified by Augustinsson.[9] Control animals were adapted either to ACh or H without injections of PMC. Each experiment was repeated three times.

PMC in doses 500 or 1,000 μg./kg. body weight markedly inhibits both adaptation to ACh (Fig. 1) and H (Table I). Four to six days after the last injection of PMC the symptoms of its action seemed to disappear, since subsequently the animals vigorously adapt as well

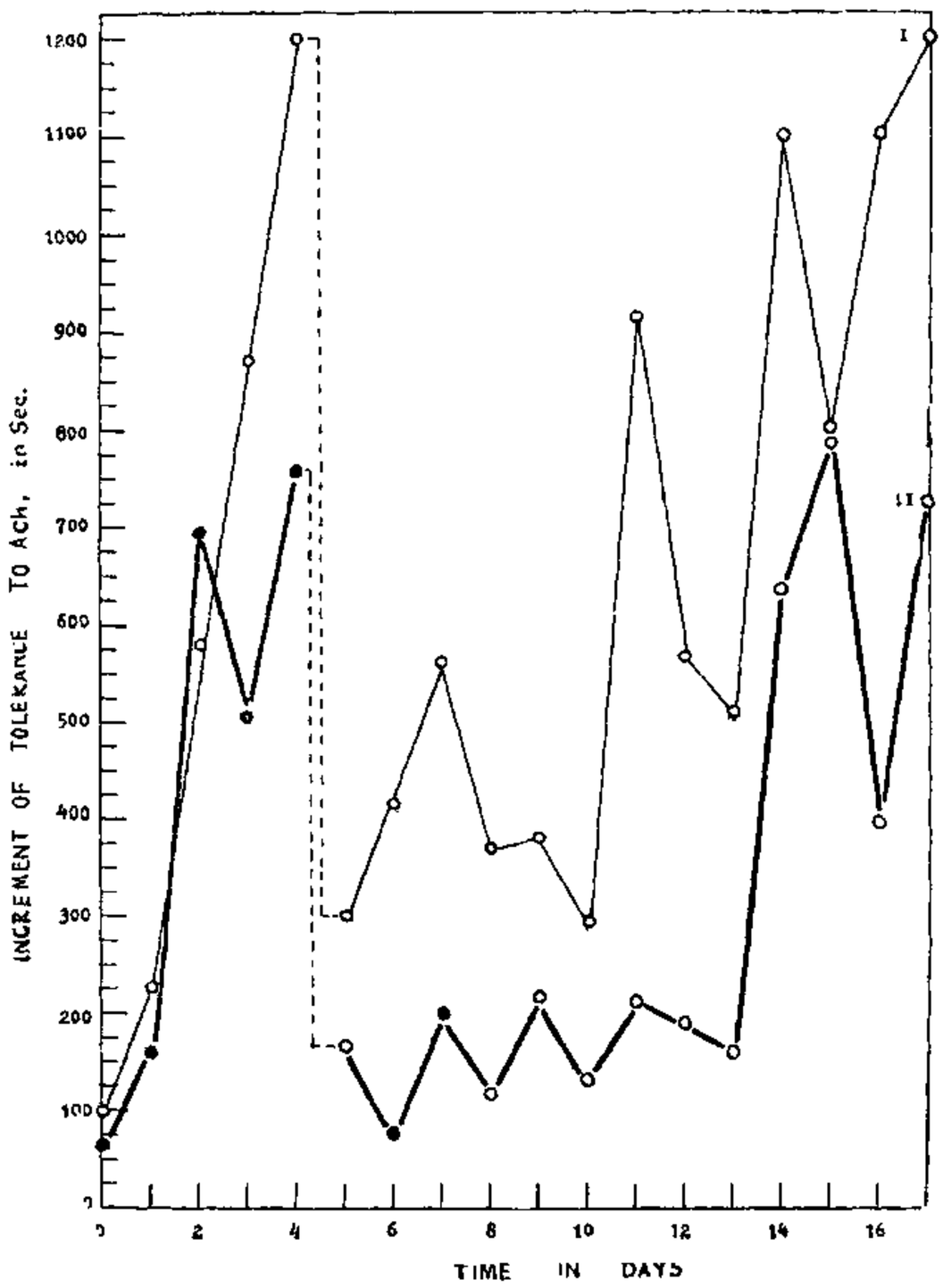

FIG. 1. The inhibitory effect of puromycin on adaptation of guinea pig to ACh. I, control; II, the animals injected seven times with PMC. Both groups I and II initially were exposed to 1·5% ACh (5 tests) and latter to 1·8% ACh; vertical dotted lines mark a moment of change of ACh concentration. Solid circles mean that PMC was injected 3-4 h Before the exposition, initially in a dose of 500μg. (kg. 5 times) and finally in a dose of 1,000μg/.kg (2 times). The experiments were carried out in September.

to H as ACh. Determinations of AChH activity in the blood revealed that Q_{AChH} did not change after 11 days of adaptation to ACh of the animals systematically injected once a day with 250 or 350 μg./kg. PMC. On the contrary, in blood of the control animals, adapted to ACh without injections of PMC, Q_{AChH} increased from initial 198 to final 302 μM ACh/60 min./ml. (Table II).

TABLE I

The effect of puromycin on adaptation of guinea-pig to 0·5% œrosol of histamine

	Group	Time of adaptation, in days											
		1	2	3	4	5	6	7	8	9	10	11	12
Tolerance to H in sec. ..	C	85	208	299	347	470	613	709	744	908	690	1100	1200
Body weight, in g. ..	C	290	293	297	300	302	303	306	310	313	315	320	325
Tolerance to H in sec. ..	PMC	77	*76*	*149*	*201*	*105*	*96*	302	311	309	314	650	735
Body weight, in g. ..	PMC	297	295	296	298	302	305	313	316	319	323	329	331

Group C = Control; group PMC = the animals were five times injected with PMC (1,000 μg./kg.) in 2-6 days of the experiment (the subsequent times of expositions are italicized). Each group contained 10 animals. These experiments were carried out in September.

TABLE II

The effect of puromycin on AChH activity in blood of guinea-pig adapted to 1·5% aerosol of ACh

Group	Number of animals	Tolerance to ACh, in sec.	$Q_{AChH} \pm$ S.D.
I ..	9	79	198 ± 12·8
C ..	12	900–1100	302 ± 10·6
PMC–250 ..	9	260	198 ± 9·5
PMC–350 ..	9	151	192 ± 10·1

I = intact animals; C = control animals adapted to ACh for 11 days; PMC–250 = 11th day of adaptation, before each exposition the animals were injected with 250 μg./kg. PMC; PMC–350 = 15th day of adaptation of the group previously noted as PMC-250, from 11th day the animals were injected with 350 μg./kg. PMC; Q_{AChH} = μM ACh hydrolysed per hour per ml of blood; blood samples were withdrawn from a heart. Determinations were carried out in June-July.

It is worthy of note that in a series presented in Fig. 1, one of the animals which was injected with 1,000 μg./kg. PMC on 6th day of the experiment did not survive this dose of the inhibitor. It died 2·5 hours after the injection showing typical symptoms of the prolonged ACh intoxication, that is convulsions and white excretion in eyeballs, despite the fact that this guinea-pig was not exposed to the aerosol this day. Since in a brain of the ACh-adapted animals the level of ACh increases up to 200%,[2] it may be supposed that this animal was intoxicated with endogenous ACh.

The data presented here indicate that (1) both adaptation to ACh and H is dependent on synthesis of protein (s), and (2) support the assumption that adaptation to ACh is dependent on ACh-induced synthesis of AChH.[2] The possibility that adaptation to H is also dependent on stimulated synthesis of AChH is now under a direct examination.

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* On leave from Institute of Experimental Pathology, Polish Academy of Sciences, Warsaw.

** On leave from Department of Plant Physiology, University of Lodz.

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