with bone resorption in the central part of the implant, so that the well-established implant, by day 15–20, consisted of a hemopoetic nodules surrounded by a thin shell of bone.

The results of chromosome studies are shown in the table and indicate that 5 weeks after implantation the majority of cell karyotypes found in implants of both marrow and spleen tissues were of recipient origin. Because both the proportion and the turnover rate of cells in hemopoietic stroma is rather low, in contrast to that of hemopoietic cells[12], one may make the reasonable assumption that these karyotypes were derived from proliferating hemopoietic cells. Furthermore the donor origin of stromal cells in similar systems, has been established by using radiation studies[13,14] or chromosome marker[6,15]. Hence, the cells of the recipient origin identified in these implants must have been different from stromal cells and were probably proliferating hemopoietic cells.

These results are in agreement with those in several other experimental systems[8,16-18], and are consistent with the view that the hemopoietic stem cell circulates but it is selectively trapped by hemopoietic stroma which provides it with a suitable milieu for proliferation and differentiation. Therefore, ectopic implants of spleen and marrow tissue may be considered as chimeric structures with the stroma of donor origin providing a framework upon which the hemopoietic stem cell of recipient origin can proliferate and differentiate[19].

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Plasma cyclic 3′,5′-guanosine monophosphate and cyclic 3′,5′-adenosine monophosphate response to methacholine in man

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Summary. Cholinergic agents are known to induce increases in tissue and plasma levels of cyclic GMP in experimental animals. We observed that i.m. injection of methacholine, a cholinergic agent, caused significant increases in plasma cyclic GMP and cyclic AMP in man.

George et al.[1] found that acetylcholine caused an increase in the level of cyclic 3′,5′-guanosine monophosphate (cyclic GMP), accompanied by no change or a slight decrease in cyclic 3′,5′-adenosine monophosphate (cyclic AMP), in the isolated rat heart. Several reports have been made of similar observations in vitro and in vivo[2-4]. Honma and Ui[5] reported that s.c. injection of cholinergic agents such as carbachol, methacholine, and bethanechol in rats caused a significant increase in plasma cyclic GMP with a slight increase in plasma cyclic AMP. Therefore the purpose of the present study is to extend from rat to man the observation that methacholine increases plasma cyclic GMP and cyclic AMP.

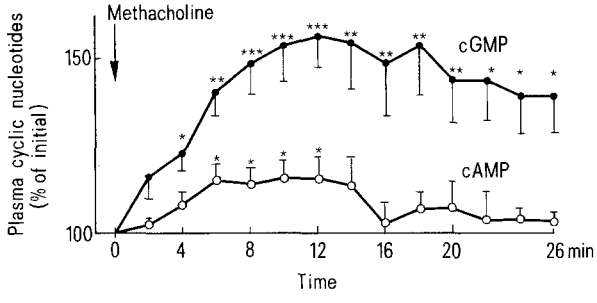
14 healthy males in excellent physical condition between the ages 18 and 42 were selected as subjects for this study. After overnight fasting and in a resting state in the supine position, all subjects received an i.m. injection of 10 mg of methacholine. Blood samples for the determination of plasma cyclic GMP and cyclic AMP were obtained via an indwelling i.v. cannula before and after the injection of methacholine at 2-min intervals for a period of 26 min. The concentrations of plasma cyclic GMP and cyclic AMP were measured by the ultrasensitive radioimmunoassay method described in a previous publication[6]. Statistical significance was determined by means of Student's t-test.

In this study in 14 normal subjects, the mean basal level of plasma cyclic GMP was 5.2 ± 0.3 pmoles/ml (mean $\pm$ SE) and the significant increases were observed between 4 min and 26 min with a peak at 12 min after the injection of methacholine. The mean basal level of plasma cyclic AMP was 20.6 ± 1.1 pmoles/ml (mean $\pm$ SE). Not only plasma cyclic GMP but also plasma cyclic AMP increased in response to methacholine and reached a peak at 12 min. After the injection of methacholine all the subjects had the symptoms referable to cholinergic stimulation such as facial flushing, salivation, sweating, palpitation, cough and a desire to urinate. The increase in plasma cyclic GMP was greater than the increase in plasma cyclic AMP, and the plasma cyclic GMP was still elevated at 26 min after the injection of methacholine, while the plasma cyclic AMP almost declined to basal level at 26 min after the injection of methacholine.

The data for the basal levels of plasma cyclic GMP and cyclic AMP in man in this study are similar to those obtained by Steiner et al.[7] using a radioimmunoassay technique.

While there are previous reports of increases in tissue and plasma cyclic GMP in response to cholinergic agents in experimental animals[1-5], the present paper is the first report that methacholine administration resulted in increases in plasma cyclic nucleotides in man. Furthermore, we observed that methacholine induced an increase not only in plasma cyclic GMP but also in plasma cyclic AMP. This result in man agrees with the report of Honma and Ui for rats[5]. However, our observation is different from those of others. George et al.[1] found that in rat heart cholinergic agents left cyclic AMP concentration unchanged, or slightly decreased it, and Lee et al.[2] made similar observations. The cause of the difference between us and others is not clear.

It is impossible to clarify what mechanisms are involved in the regulation of plasma cyclic nucleotides in response to methacholine in man from our limited data, but it is likely that different mechanisms participate in the regulation of plasma cyclic GMP and cyclic AMP, because there were some differences between the patterns of increment of plasma cyclic GMP and cyclic AMP as shown in our study. It has been generally accepted that the levels of tissue and plasma cyclic GMP and cyclic AMP are under separate regulatory control. Cyclic AMP is considered to be closely related to beta-adrenergic receptors[8] and a recent observation, reported by Leveston et al.[9], that cholinergic stimulation releases endogenous cathecholamines in human plasma, may explain the observed increments in plasma cyclic AMP in our study. On the other hand, since the increases in tissue and plasma level of cyclic GMP in animals induced by cholinergic agents were antagonized by atropine, a muscarinic agent, cyclic GMP is considered to be involved in the action of cholinergic functions[1,2,5], which may explain the observed increments in plasma cyclic GMP in our study. Anyhow, further studies are required to determine what mechanisms are involved in the regulation of the response of plasma cyclic nucleotides to cholinergic agents in man.



Effect of methacholine on plasma cyclic GMP and cyclic AMP in normal subjects. 10 mg of methacholine was injected i.m. into 14 healthy adults at time-0 and increases in plasma cyclic GMP (●—●) and cyclic AMP (○—○) were plotted as a percentage of the initial value. The values shown are means ± SE. The initial values for cyclic GMP and cyclic AMP were 5.2 ± 0.3, 20.6 ± 1.1 pmoles/ml (mean ± SE) of plasma respectively. Significant increases in plasma cyclic nucleotides were observed $*p < 0.05$, $**p < 0.02$, $***p < 0.01$.

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Giant granules in adrenaline-secreting chromaffin cells of lizard adrenal glands after metyrapone administration[1]

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Summary. Secretory granules of extraordinary size, some of them bigger than the cell nucleus, abound in the adrenaline cells of lizard adrenals after metyrapone injections during 7 days. In these granules, the bounding membrane is studded with ribosomes, and the core is formed by rounded small subunits. Some granules of this type are also found in noradrenaline cells. They may represent an exceptionally increased elaboration and storage of adrenaline, induced by metyrapone probably through its action on steroidogenic tissue.

The association of chromaffin and steroidogenic tissues in the adrenal gland of vertebrates is not casual. In the lizard adrenal, which jointly contains adrenaline- and noradrenaline-producing chromaffin elements, only cells contiguous to interrenal - or cortical - tissue are of the adrenaline-secreting type[3]. So, this gland gives a morphological demonstration of the role that corticosteroids play in the formation of phenylethanolamine-N-methyl transferase (PNMT), the enzyme that allows the synthesis of adrenaline, starting from noradrenaline[4]. In turn, chromaffin tissue was shown to be able, at least in mammals, to perform various steps in steroid biosynthesis[5].

The present observations refer to ultrastructural changes produced in the chromaffin tissue of lizard adrenal by the effect of metyrapone, a substance that specifically interferes steroidogenesis, inhibiting 11β-hydroxylation[6] and, in certain conditions, the cleavage of cholesterol to pregnenolone[7,8]. The action of metyrapone on the structure of interrenal tissue and several other endocrine glands of the same species of lizard has been already studied[9-12].

Material and methods. 8 adult male specimens of the teiid lizard *Cnemidophorus lemniscatus* (L.), with snout-vent lengths between 7 and 8 cm, were captured in July and maintained in natural photoperiod and temperature. 4 animals received daily i.p. injections of 2.5 mg of metyrapone (metopirone bitartrate CIBA), 1% in aqueous solution, during 7 days. The remaining lizards were given similar injections, but of saline solution, and served as controls.