The differential response of the different explants in the same medium may be due to changes in the physiological conditions of the material. Although there is evidence that polyploid cells may arise from endoreduplicated nuclei in the original explant, the range of chromosome number and structure observed in established cultures strongly points to the origin of these changes during culture. In the present investigation tetraploid cells occur at maximum frequency in seed calluses, while a tendency toward diploidy was found in leaf calluses. Although Partanen[5,6] reported that chromosomal instability in culture depends upon the type of plant parts from which the callus originated, the present investigations show variation in chromosome number in all the 3 different explants cultured in the same medium.

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Preservation of *Bordetella pertussis*

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Summary. Bordetella pertussis has been shown to remain viable for a period of 13 years on freeze-drying. The revived cultures had unaltered antigenic composition and biological activity.

Frequent subculture of a microbial species is liable to result in change of antigenic and other characters. Therefore, maintaining viability of an organism for longer periods without resorting to repeated subcultures is highly desirable. Freezing at −70 °C with 15% glycerol was found to be a satisfactory means of preservation of *Treponema pallidum* and other bacteria[2]. The factors affecting the death rate of *Escherichia coli* at temperatures between −1.5 °C and −195 °C have been studied[3]. Reports of the preservation of *B. pertussis* for 45 months in 15% glycerol at −70 °C and of freeze-dried cultures for 10 years are available[4,5]. The present communication reports the preservation of *B. pertussis* for a period of 13 years.

Materials and methods. Strains 134 and 509 routinely used by us for the manufacture of pertussis vaccine were suspended separately either in Lomodex (10% dextran in 5% dextrose, Rallis India Ltd) or aqueous gum acacia solution. 0.2 ml of the culture suspension containing approximately 200×10^9 organisms/ml were freeze-dried in 100×8 mm sterile glass tubes and sealed under vacuum. To study the effect of an inert gas on the viability of cultures, gaseous nitrogen was passed into some of the tubes before sealing. All tubes containing freeze-dried cells were stored at 4–10 °C until used. The viability of the cultures was tested on duplicate tubes of Bordet-Gengou (BG) medium. Tubes were incubated at 35 °C for a maximum of 96 h. Antigenic composition of BG-grown cultures was checked with *B. pertussis* monospecific factor 1, 2, and 3 sera by slide agglutination method[6]. Test for dermonecrotic activity was performed in rabbits. 0.1 ml of a suspension containing 1.0×10^9 organisms/ml was inoculated intradermally. Results were recorded at 48 and 72 h. The maximum reaction obtained has been reported.

Result and discussion. The findings have been summarized in the table. A freeze-dried culture under vacuum showed viability upto 13 years – the maximum period of observation in this study. Revived cultures of strain 134 agglutinated with factors 1 and 3 and those of strain 509 with factor 1, 2, and 3 sera. This is in agreement with the known antigenic composition of the 2 strains. The cultures from the

Viability and other characteristics of *B. pertussis* cultures after various periods of freeze-drying

Strain	Date of freeze-drying	Conditions of freeze-drying		Growth up to 96 h at 35 °C		Agglutination with factor sera			Dermonecrotic reaction (mm)
		Suspending fluid	Gaseous condition	BG Tube I	BG Tube II	1	2	3	
509	March, 66	Gum acacia	Vacuum	+	+	+	+	+	10×10
509	July, 72	Gum acacia	Vacuum	+	+	+	+	+	15×10
509	July, 72	Lomodex	Nitrogen	−	−				
509	April, 76	Lomodex	Vacuum	+	+	+	+	+	10×10
509	April, 76	Lomodex	Nitrogen	−	−				
509	April, 79	Lomodex	Vacuum	+	+	+	+	+	15×10
134	July, 66	Gum acacia	Vacuum	+	+	+	−	+	15×10
134	July, 72	Gum acacia	Vacuum	+	+	+	−	+	15×10
134	July, 72	Lomodex	Nitrogen	−	−				
134	April, 76	Lomodex	Vacuum	+	+	+	−	+	20×10
134	July, 76	Lomodex	Nitrogen	−	−				
134	April, 79	Lomodex	Vacuum	+	+	+	−	+	15×10

+ = Growth, agglutination; − = no growth, no agglutination.

tubes of freeze-dried material stored for different durations showed dermonerotic activity in rabbit skin ranging from 10.0× 10.0 mm to 20.0× 10.0 mm. Thus, these cultures were found to maintain not only viability, but also there was no alteration in their antigenic composition. No difference in dermonecrotic activity between the older and freshly freeze-dried cultures was observed. Tubes under the atmosphere of gaseous nitrogen, however, showed scanty growth immediately after freeze-drying and no growth in older cultures. Since these tubes did not yield growth at the time of final testing their antigenic composition and dermonecrotic activity could not be determined. In our experience both Lomodex and aqueous gum acacia work very well as suspending fluids for freeze-drying.

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In vitro characterization of adrenergic receptors mediating extrusion of preformed sebum from preputial gland of rat[1]

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Summary. Extrusion of preformed sebum from the preputial glands of rat after phenylephrine and adrenaline treatment, and its inhibition by α-receptor blocking agents under in vitro conditions, shows that secretory response of the glands is influenced by α-adrenergic receptors, and isoproterenol - a β-agonist - is not effective in elicitation of exudation of secretion from the preputial gland.

There is neurohistochemical evidence that the preputial gland, a sebaceous analogue, of rat is supplied by adrenergic as well as cholinergic nerves[2]. It is not definitely known to what extent the sebaceous gland is under the control of nervous system. Neurohistological studies have yielded no evidence of secretory innervation for the sebaceous gland. Serrati[3] thought that vegetative nervous system regulates sebaceous secretion, basing his conclusion on the observations on patients with various neuronal disorders. Savill[4] stated that the sebaceous glands of the skin are under the control of autonomic nervous system. Nexmand[5] observed seborrhea following complete trans-section of the facial nerve, while Hodgson-Jones et al.[6] noted no change in sebaceous secretion in denervated area of skin. Starling[7] stated that sebum is squeezed out by intradermal injections of epinephrine, but Kligman and Shelley[8] believed that Starling's observations were erroneous as they could not find any expulsion of preformed sebum after either epinephrine or acetylcholine administration. Present experiment is an attempt to observe the effects of adrenergic agents under in vitro conditions, so as to probe deeper into the study of involvement of neurotransmitters in the release of preformed sebum from the preputial glands of rat.

Material and methods. Wild rats *(Rattus rattus)* were used in the present study. The amount of preformed sebum is greater in wild rats than that in albino rats. When the amount of preformed sebum is greater, it is easy to detect its extrusion visually. For this purpose wild rats were selected. The preputial glands were removed and immersed in 20 ml of oxygenated Ringer's solution. After 20 min of exposure to Ringer's solution; adrenaline, isoproterenol or phenylephrine were added to Ringer's solution separately at concentrations of 1×10^{-5} moles/20 ml in each case. Extrusion of the sebum was considered as the positive response. In another set of experiments, 3 different blocking agents viz., propranolol (β-blocking agent), dibenamine and phentolamine (α-blocking agents) at 1×10^{-4} moles/20 ml concentration were added. Preputial glands were exposed to blocking agents for 30 min. After 30 min, adrenaline was added and extrusion of secretion that followed was considered as the positive response.

Results. The table represents the pattern of responses of preputial glands to various drugs employed during the course of present study. Addition of adrenaline or phenylephrine to the Ringer solution caused quick extrusion of secretion from the duct and the response was apparent over a considerable period of time. Isoproterenol addition did not elicit such a positive response.

Later, the glands were preincubated for 30 min with blocking agents viz., dibenamine, phenotolamine or propranolol, with a view to differentiate more precisely between α- and β-receptor-mediated responses. At the end of 30 min of preincubation, adrenaline was added in each case. It was observed that the glands preincubated with α-receptor blocking agents - dibenamine or phentolamine - did not respond, while those preincubated with β-receptor blocking agent - proprenolol - still showed a positive response to addition of adrenaline.

Discussion. Skin glands of lower vertebrates (anurans) are reported to have α-adrenergic receptor-mediated secretory response[9]; while cutaneous and hedonic glands of the red-spotted newt are reported to respond to cholinergic stimu-

In vitro response of the preputial gland of rat to adrenergic agonists and antagonists

Experimental group treated with	Response
Adrenaline	+
Phenylephrine	+
Isoproterenol	−
Dibenamine + adrenaline	−
Phentolamine + adrenaline	−
Propranolol + adrenaline	+

+ Sign in the column of response indicates positive response to the drug whereas − sign indicates negative response.