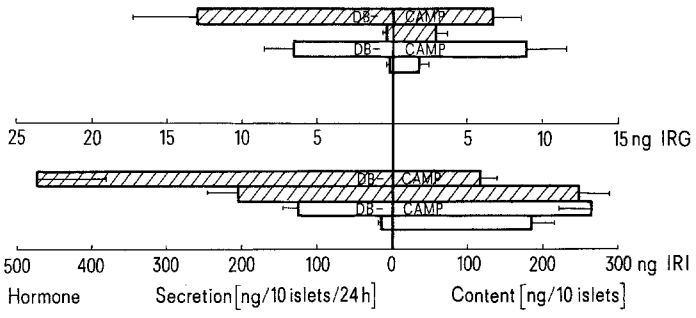

Fig. 2. Influence of 2 m*M* dibutyryl-cyclic AMP (DB-CAMP) on hormone storage and release (7th–8th day) of rat islets cultured for 8 days at 5 m*M* glucose (□) or 15 m*M* glucose (▨). Effect of DB-CAMP on insulin (IRI) and glucagon (IRG) release $p < 0.01$ at 5 and 15 m*M* glucose. Effect of DB-CAMP on glucagon storage at5 m*M* glucose $p < 0.02$. Values as mean ± SEM for 7 tissue preparations.

acids, it seems likely that both cell types show an equal behavior with respect to substances which enhance the intracellular cyclic-AMP level as hypothesized by GERICH et al.[16] and HOWELL et al.[8].

The present results indicate that it is also possible to induce hormone biosynthesis and release in cultured A- and B-cells, even if hormone release and storage are already diminished. Cyclic AMP can probably modulate the hormone release and prevent the further drop of specific cell functions in cultureds islets.

Zusammenfassung. Insulin- und Glukagonsekretion sowie der Hormongehalt kultivierter Langerhans'scher Inseln der Wistarratte wurden nach Gabe von 2 m*M* Dibutyryl-cycl. AMP (DB-CAMP) in Gegenwart von 5 m*M* bzw. 15 m*M* Glukose bestimmt. DB-CAMP steigert sowohl die Sekretion als auch den Glukagongehalt der A-Zellen, während die B-Zellen bei unveränderter Speicherfähigkeit durch eine höhere Hormonabgabe gekennzeichnet sind. Die Insulinsekretion kultivierter Inseln, die 4–6 Tage bei 5 m*M* Glukose inkubiert wurden, konnte auch durch Erhöhung der Glukosekonzentration auf 15 m*M* gesteigert werden.

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The Effect of Short-Term Treatment of Low Dose of Methallibure (ICI Compound 33,828) on the Testis and Thumb Pad of Skipper Frog, *Rana cyanophlyctis* (Schn.)

The antigonadotropic effect of Methallibure has been well established in mammalian species[1], while comparative studies on lower vertebrates are limited to a few species only[2–6]. The present work was undertaken to investigate the effects of low dose of Methallibure on the testis, with reference to spermatogenesis and the steroidogenic activity of the interstitial Leydig cells, and the androgen-dependent thumb pads of skipper frog, *Rana cyanophlyctis*.

Adult male specimens of *R. cyanophlyctis* were obtained from the surrounding areas of Dharwar, and were divided into 2 groups. The first group specimens (10) were injected with saline (0.65%) only, to serve as the controls. The second group specimens (10) were injected with saline suspension of Methallibure, biweekly for 4 weeks. The total dose being 10 mg for each of the experimental frog. All the frogs were autopsied 3 days after the last injection. The relative testis weights were recorded and representative pieces of testes and thumb pads were fixed in Bouin's fluid for histological and histometric studies. The remaining pieces of testes were used for the histochemical assay of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities as described earlier[7] and also for the quantitative determination of cholesterol content[8].

It is evident from the Table I that there is no appreciable effect on the average testis-weight, testis diameter and the tubule diameter due to short-term treatment with low dose of Methallibure. Similarly, no marked alteration in the spermatogenetic activity was observed. There was, however, significant decrease ($p < 0.001$) in the Leydig cell nuclear diameter (Table I) in the treated specimens. Further, in controls the Leydig cell nuclei appeared round in outline and contained coarse chromatin granules, and also exhibited abundant Δ^5-3β-HSDH and G-6-PDH enzyme activities histochemically, whereas in treated specimens the Leydig cell nuclei appeared flattened and contained fine chromatin granules and also exhibited decreased Δ^5-3β-HSDH and G-6-PDH activities (Table I), with the concomitant rise in the total cholesterol (18%) content (Table II). The height of epidermis and the

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Table I. Effect of Methallibure on the testis of *R. cyanophlyctis*

Group	Average testis Wt. (mg/100 g body Wt. ± SE)	Average diameter (μm ± SE)			Δ^5-β HSDH activity[a]	G-6-PDH activity[a]
		Testis	Testis-tubule	Leydig cell nucleus		
Control	260.94 ± 34.7	1858.5 ± 25.2	251.7 ± 10.18	4.48 ± 0.01	++	++++
Experimental	249.76 ± 25.37 $p > 0.5$	1748.2 ± 77.8 $p < 0.3$	235.7 ± 4.44 $p < 0.3$	4.05 ± 0.06 $p < 0.001$	+	++

SE, Standard error. [a] Intensity of reaction is visually graded from (+) to (++++); p-values calculated by Student's t-test between control and experimental groups.

Table II. Effect of Methallibure on the testis cholesterol in *R. cyanophlyctis*

	μg/100 mg wet weight of testis		Increase over control value (%)
	Control	Experimental	
Free cholesterol	440.43	447.10	1.51
Total cholesterol	559.28	763.00	18.54

Table III. Effect of Methallibure on the thumb pad of *R. cyanophlyctis*

Group	Average height μm ± SE	
	Epidermis	Glandular epithelium
Control	78.05 ± 7.77	24.38 ± 2.76
Experimental	63.55 ± 9.00 $p < 0.4$	11.93 ± 0.64 $p < 0.01$

SE, standard error. p-values calculated by Student's t-test between control and experimental groups.

glandular epithelium of thumb pads decreased markedly (Table III) in the treated specimens. The epidermis was less papillate and the mucous glands were atrophic in the treated specimens. These observations suggest an impaired androgen production by the testes which is reflected in the regression of the thumb pad, an androgen dependent secondary sexual character. The present findings are in conformity with those reported earlier on other species[1-5] wherein Methallibure was found to cause regression of the secondary sex characters in male. However, the low dose of Methallibure used did not significantly influence the testicular histology and histometry during the short-term treatment.

Zusammenfassung. Die Behandlung des männlichen Frosches *Rana cyanophlyctis* mit Methallibur (ICI Verbindung 33, 828) während der Dauer von vier Wochen ergab die folgenden Wirkungen: 1. Rückbildung der Leydigschen Zellen und Abnahme ihrer Δ^5-3β-HSDH-Aktivität, 2. Anstieg des Totalgehaltes an Cholesterol, und 3. Rückbildung der Daumenschwielen. Bedeutende histologische und histochemische Veränderungen der Samenkanälchen wurden jedoch nicht beobachtet.

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[9] Supported by a grant No. 4756 from the U.G.C. of India.
[10] Supported by a grant No. M66.117 from the Population Council, Bio Medical Division, New York.
[11] We are grateful to Imperial Chemical Industries Limited, England, for the free gift of Methallibure and to Dr. M. A. Rao for providing laboratory facilities.

Effect of Pimozide, a Dopaminergic Blocking Agent, on Hypothalamic Luteinizing Hormone Releasing Hormone Activity in Hypophysectomized Rats

Hypothalamic releasing factor (RF) mechanisms appear to be regulated by diencephalic dopaminergic systems[1,2]. RF activity has been detected in the plasma of hypophysectomized animals[3,4], thus providing a model in which the effects of pharmacologic agents on the hypothalamic neurotransmitter/RF function can be evaluated. In view of our recent report[4] describing the elimination of plasma luteinizing hormone (LH) releasing hormone (LRF) activity in hypophysectomized rats with the

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