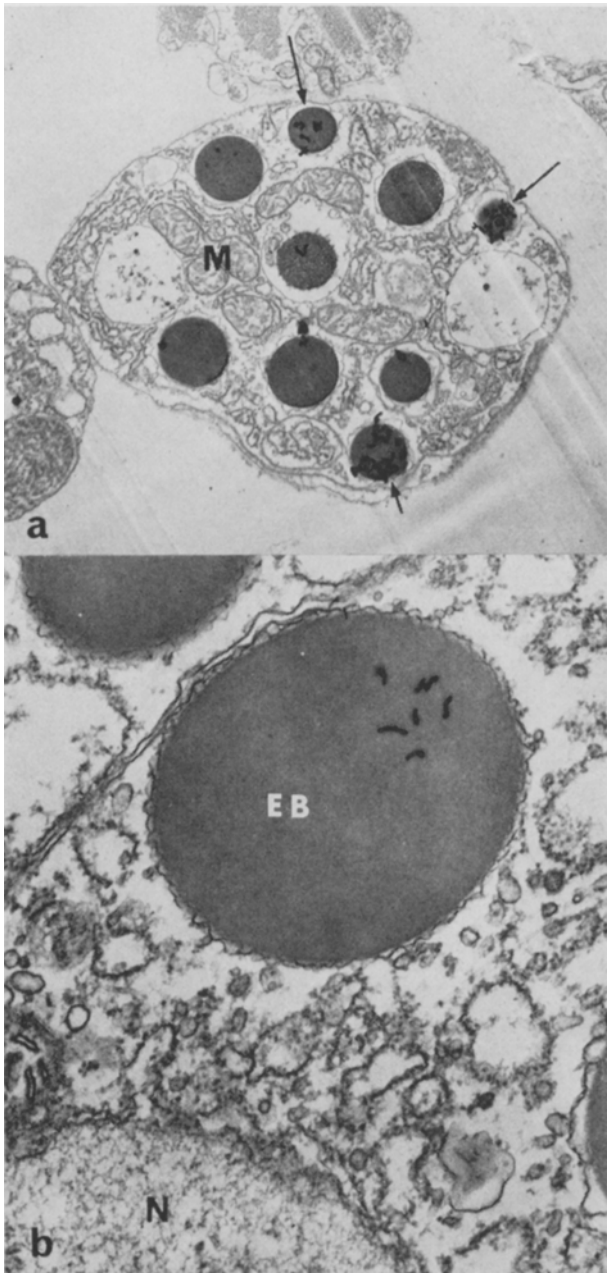


Fig. 2. a) Electron microscopic autoradiograph showing an iodine-binding blood cell which contains several electrondense bodies in its cytoplasm. Label (see arrows) is always associated exclusively with these electron-dense bodies. ×6,000. b) Enlargement of one of the electron-dense bodies which contains radioactive iodine. ×50,000. EB, electron-dense body; M, mitochondria; N, nucleus.

and postabdomen. These cells contain several membrane-bound electron-dense bodies in which the $^{125}I$ is found (Figures 2a and b).

In vertebrates, similar membrane-bound electron dense bodies are found in the cells that make up the follicular epithelium of the thyroid gland[11–13]. The number and size of these electron dense bodies is related to the functional demands that are placed on the thyroid gland. Autoradiographic studies show that iodine is bound by these electron dense bodies[13].

Membrane-bound electron dense bodies have also been observed in the cells that make up zone 7 of the ascidian endostyle[14,15]. In specimens that have been exposed to $^{125}I$ for a short period of time, silver grains have been observed over the apical surface and the multivesicular bodies of the zone 7 cells. Incubation for a longer period of time causes the transfer of the silver grains to the membrane-bound electron dense bodies in the same cells[14,15]. These are the cells of the endostyle where thyroxine biosynthesis is thought to occur.

The structural similarity of the membrane-bound electron dense bodies in the thyroid epithelium of vertebrates, the zone 7 endostyle cells and the iodine binding blood cells of ascidians, suggests that iodine-binding blood cells might elaborate thyroid hormones.

*Zusammenfassung.* Gewisse Blutkörperchen in den Oozoiden von *Amaroucium constellatum* nehmen $^{125}I$ in grosser Menge auf. Das Cytoplasma dieser Zellen besitzt membraneingewickelte elektronendichte Körperchen, wo sich die Silberkörner ausschliesslich befinden. Die Möglichkeit der Biosynthese des Schilddrüsenhormons in solchen Blutkörperchen wird diskutiert.

M. FUKUMOTO[16]

*Biological Institute, College of General Education, Nagoya City University, Mizuho-ku, Nagoya 467 (Japan), 18 November 1974.*

[11] H. SHELDON, J. M. MCKENZIE and D. VAN NIMWEGAN, J. Cell Biol. *23*, 200 (1964).
[12] R. COLEMAN, P. J. EVENNET and J. M. DODD, Gen. comp. Endocr. *10*, 34 (1968).
[13] P. NEUENSCHWANDER, Z. Zellforsch. *130*, 553 (1972).
[14] A. THORPE, M. C. THORNDYKE and E. J. W. BARRINGTON, Gen. comp. Endocr. *19*, 559 (1972).
[15] A. D. DUNN, J. exp. Zool. *188*, 103 (1974).
[16] The author wishes to express his sincere thanks to Dr. G. FREEMANN for providing the facilities where this work was done and for reading this manuscript. He is also indebted to Dr. A. JACOBSON for the use of his electron microscope facility and to Dr. S. FULLILOVE for technical assistance. This work was carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts and the Department of Zoology at the University of Texas at Austin. It was supported by N.I.H. grant No. GM 20024-02.

## Effect of Dibutyryl Cyclic AMP on Glucagon and Insulin Storage and Secretion in Organ Culture of Rat Islets

It is generally accepted that substances which enhance the intracellular level of cyclic AMP increase insulin release from endocrine pancreas[1–3]. It has also been suggested that changes of the cyclic-AMP value may be involved in glucagon secretion[4–8]. We have only a few results with respect to the cultured endocrine pancreas[2,7], especially to cultured islets, which are characterized by a continuous diminution of release and storage of hormone with the duration of cultivation[9–11].

The aim of this paper is to investigate the effect of $N^6$-2′-*O*-dibutyrylcyclic adenosine-3′,5′-monophosphate (DB-CAMP) on the content and secretion of hormones of cultivated rat islets. Collagenase isolated islets of adult Wistar rats (starved overnight with a body weight of

160–180 g) were cultivated as described[11,12] in groups of 10 islets on glass fibre vlies in 2 ml modified TCM 199 medium (Difco Laboratories Detroit, USA) containing 1 mg hydrocortisone/l and supplemented with 5% fetal and 5% heat inactivated calf serum (called complete medium).

The islets were incubated in complete medium containing 5 m*M*, 15 m*M* glucose alone or together with 2 m*M* DB-CAMP (C. F. Boehringer & Söhne, GmbH, Mannheim, BRD). In other experiments the islets were at first cultivated at 5 m*M* glucose for 4 or 6 days and then given 15 m*M* glucose alone or with 2 m*M* DB-CAMP. At the change of medium (1, 2 or 3 days interval) insulin and glucagon were determined radioimmunologically[13,14]. The content of hormone in the islets was measured after lyophilization[11]. The results are calculated as ng/10 islets/24 h (secretion) or ng/10 islets (content) and are given as mean ± SEM with the number of observations in parentheses. The significance was checked by *t*-test.

Influence of dibutyryl-cyclic AMP (DB-CAMP) on the insulin (IRI) and glucagon (IRG) release from islets cultivated at 5 m*M* glucose

| Age of culture (days) | DB-CAMP (m*M*) | IRI (ng/10 islets/24 h) | IRG (ng/10 islets/24 h) |
|---|---|---|---|
| 2 | – | 21.5 ± 3.09 | 1.2 ± 0.18 |
| | 2 | 165.7 ± 28.28[a] | 1.1 ± 0.35 |
| | – | 23.8 ± 2.95 | 1.9 ± 0.22 |
| 3 | 2 | 192.6 ± 45.97[a] | 2.9 ± 0.66 |
| | – | 25.2 ± 3.70 | 1.0 ± 0.28 |
| 4 | 2 | 137.4 ± 13.66[a] | 3.3 ± 0.72[a] |
| | – | 10.8 ± 1.74 | 0.1 ± 0.03 |
| 7 | 2 | 73.0 ± 11.99[a] | 3.3 ± 0.90[a] |
| | – | 15.6 ± 2.42 | 0.2 ± 0.04 |
| 8 | 2 | 124.2 ± 20.01[a] | 6.6 ± 1.93[a] |

Values as mean ± SEM for 7–15 tissue preparations. [a] $P < 0.01$: Effect of DB-CAMP on hormone release at 5 m*M* glucose.

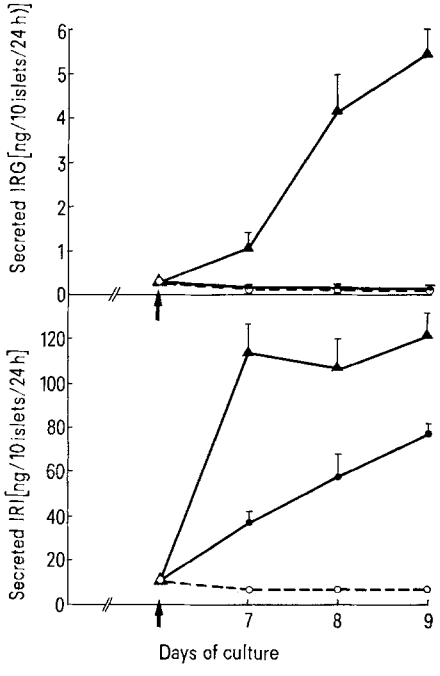


Fig. 1. Insulin (IRI) and glucagon (IRG) release from cultivated rat islets. Groups of 10 islets were cultivated at 5 m*M* glucose for 4 or 6 days and then were given 5 m*M* glucose alone (○---○), 15 m*M* glucose alone (●—●) or 15 m*M* glucose with 2 m*M* dibutyryl-cyclic AMP (▲—▲). Values as mean ± SEM for 8–10 culture bottles from 2 tissue preparations. ↑, Control value before 15 m*M* glucose and 2 m*M* DB-CAMP were given. Effect of DB-CAMP on insulin and glucagon release on 7th, 8th and 9th day: $p < 0.01$.

As is shown in the Table and Figures 1 and 2, 2 m*M* DB-CAMP significantly increase the insulin and glucagon release from cultured rat islets both at low and high glucose concentrations and independent of the day of stimulation (at the beginning of cultivation: Table and Figure 2 or on 4th or 6th day: Figure 1). It should be stressed that it is also possible to induce, by enhancement of the glucose concentration alone on 4th or 6th day, a continuous increase of insulin release as shown in Figure 1, although the insulin content after such a time of culture is only about 50% that of freshly isolated islets[11].

In contrast to our results, the insulin release of at 3.3 m*M* or 6.1 m*M* cultured mice islets could not be stimulated with 16.7 m*M* glucose alone or with theophylline in short time experiments[15], and a long-term effect of the glucose challenge after some days of culture must be discussed.

DB-CAMP elevated the insulin secretion at 5 m*M* and 15 m*M* glucose but the effect at high glucose was not so drastical (Figure 2). The stimulating effect of DB-CAMP on insulin and glucagon release was accompanied by an augmented storage of glucagon in the cultured islets (Figure 2) whereas the insulin content of islets was generally not influenced by DB-CAMP. The drastical effect of DB-CAMP on hormone release and storage suggests not only an effect on the secretion but also on the biosynthesis. This is supported by the calculation of the amount of hormone release during the last 24 h and content on the 8th day as indicated in Figure 2.

Furthermore, the results show that in B-cells the hormone release is more influenced than the storage, especially at 15 m*M* glucose (Figure 2) and that 2 m*M* DB-CAMP in presence of 5 m*M* glucose have nearly the same effect as 15 m*M* glucose alone. Despite the known differences between the hormone release from A- and B-cells in regard to glucose, glucose metabolites and fatty


[1] W. J. Malaisse, F. Malaisse-Lagae and P. H. Wright, Endocrinology *80*, 99 (1967).
[2] A. E. Lambert, Y. Kanazawa, I. M. Burr, L. Orci and A. E. Renold, An. N.Y. Acad. Sci. *185*, 232 (1971).
[3] W. Montague and J. R. Cook, Biochem. J. *122*, 115 (1971).
[4] T. Mc. C. Chesney and J. G. Schofield, Diabetes *18*, 627 (1969).
[5] V. Leclercq-Meyer, G. R. Brisson and W. J. Malaisse, Nature, Lond. *231*, 248 (1971).
[6] G. Rosselin, C. Jarrousse, F. Rancon and B. Fortha, C. r. Acad. Sci., Paris *276*, 1017 (1973).
[7] E. B. Marliss, C. B. Wollheim, B. Blondel, L. Orci, A. E. Lambert, W. Stauffacher, A. A. Like and A. E. Renold, Eur. J. clin. Invest. *3*, 16 (1973).
[8] S. L. Howell, J. C. Edwards and W. Montague, Horm. Metab. Res. *6*, 49 (1974).
[9] S. Moskalewski, Gen. comp. Endocr. *5*, 342 (1965).
[10] A. Andersson and C. Hellerström, Diabetes *21*, Suppl. 2, 546 (1972).
[11] B. Ziegler, R. Butter, M. Ziegler, H.-J. Hahn and H. Fiedler, Acta biol. med. germ. *32*, 503 (1974).
[12] B. Ziegler, R. Butter, H.-J. Hahn, R. Mehling and H. Fiedler, Experientia *29*, 881 (1973).
[13] M. Ziegler, U. Karg, J. Gens, B. Johannsen, R. Michael and D. Michaelis, VIII. Nuklearmed. Symp. Reinhardsbrunn (1971), p. 141.
[14] M. Ziegler, R. Michael, H. E. Stein and D. Klatt, Radiobiol. Radiother. *15*, 79 (1974).
[15] A. Andersson, Diabetologia *10*, 357 (1974).

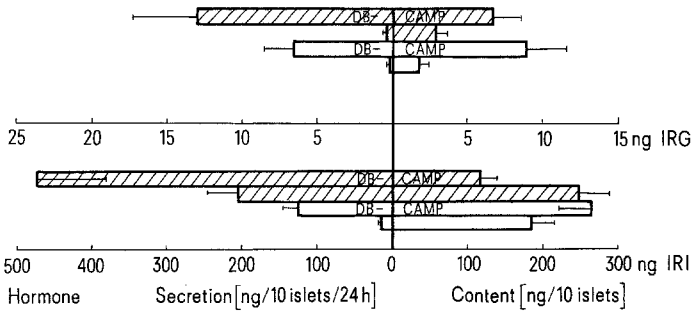


Fig. 2. Influence of 2 m*M* dibutyryl-cyclic AMP (DB-CAMP) on hormone storage and release (7th–8th day) of rat islets cultured for 8 days at 5 m*M* glucose (□) or 15 m*M* glucose (▨). Effect of DB-CAMP on insulin (IRI) and glucagon (IRG) release $p < 0.01$ at 5 and 15 m*M* glucose. Effect of DB-CAMP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

BRIGITTE ZIEGLER, M. ZIEGLER and RENATE MEHLING

*Zentralinstitut für Diabetes, Forschung und Behandlung 'Gerhardt Katsch', DDR–2201 Karlsburg/Greifswald (German Democrat Republic, DDR), 11 November 1974.*

[16] J. E. GERICH, M. LANGLOIS, C. NOACCO, V. SCHNEIDER and P. H. FORSHAM, J. clin. Invest. *53*, 1441 (1974).

## 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 $\Delta^5$-3$\beta$-hydroxysteroid dehydrogenase ($\Delta^5$-3$\beta$-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 $\Delta^5$-3$\beta$-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 $\Delta^5$-3$\beta$-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

[1] G. E. PAGET, A. L. WALPOLE and D. N. RICHARDSON, Nature, Lond. *192*, 1191 (1961).
[2] W. S. HOAR, J. WIEBE and E. HUI WAI, Gen. comp. Endocr. *8*, 101 (1967).
[3] J. P. WIEBE, Can. J. Zool. *46*, 751 (1968).
[4] S. PANDEY and J. F. LEATHERLAND, Can. J. Zool. *48*, 445 (1970).
[5] R. K. RASTOGI, G. CHIEFFI and C. MARMORINO, Z. Zellforsch. *123*, 430 (1972).
[6] S. R. KANAKRAJ and N. S. GANGADHAR, Gen. comp. Endocr. *8*, 72 (1967).
[7] S. K. SAIDAPUR and V. B. NADKARNI, Indian J. exp. Biol. *10*, 425 (1972).
[8] B. L. OSER, in *Hawk's Physiological Chemistry*, 14th edn. (McGRAW HILL Company 1965), p. 1062.