Action of Insulin on the Glycogen Content of Liver and Muscle of the Murrel, *Ophicephalus striatus* (Bloch)

It is generally known that the action of insulin and other hormones is the same in fish as in higher vertebrates. Earlier the author[1-4] has pointed out that in many details the action of insulin differs from that in other vertebrates. Further it has been shown also that hypoglycemia is produced at different levels with different doses of insulin[4]. Very heavy doses are found to be lethal, producing the minimum blood glucose. The next question which arose was what has happened to the disappeared glucose? With this in view, glycogen content, amino nitrogen and amino acids in the different tissues have been studied. The only reference that is available on this subject with regard to fish is that of ROOT et al.[5] who studied the glycogen content of liver following insulin injections.

In the present account, the action of insulin on the glycogen content of liver and muscle has been described for *Ophicephalus striatus* treated with different doses of insulin in the same manner in which the blood glucose was studied[4]. Detailed experimental plans have been reported earlier[1-4]. Following 5 international units (IU) of insulin at an interval of $^1/_2$ h for 3 h fish were autopsied for the assaying liver and muscle glycogen. They were also autopsied at 4, 12, 24, 36 and 48 h respectively when 7.5, 10, 15, 20 and 40 IU of insulin were injected. 60 IU of insulin being lethal, the autopsy was done just at the time of death of the fish which ranged from 32 to 45 h. For each experimental group, 5 fish were kept.

For quantitative determination of glycogen, colorimetric micro-method developed by KEMP and KITS[6] has been employed using the photo electric colorimeter with 520 mμ filter. Standard reference curves were prepared with known strength of glycogen solutions. Glycogen content was expressed in mg/g of tissue wet weight. Animals injected with distilled water in the place of insulin served as control.

The normal level of glycogen of liver is 12.36 $\pm$ 0.18 mg/g of tissue (Figure, *N*). Injection of 5 IU of insulin produces a maximum increase in liver glycogen of about 43% at 1.5 h after injection, and the normal level was reached at the end of 3 h. When compared with normal, the rise is significantly high at all stages (Figure, A). 7.5 IU of insulin increases 100% liver glycogen and at higher doses the glycogen level rises several fold. During convulsions following 40 IU and at the close of death following 60 IU of insulin, the rise is 4.7 and 5.7 times the normal (Figure, C).

On the other hand, muscle glycogen shows an increase of 114% at 1.5 h following 5 IU of insulin injection. But significant rise is observed only at the end of 1 h and similarly the return to the normal level is attained at 2 h itself. The values at 0.5 and 2.5 h are not significantly different from that of normal (Figure, B). Marked rise in the muscle glycogen, much more than in liver, is observed during higher doses of insulin treatment. 7.5 IU of insulin produces an increase of 181% and 40 and 60 IU induce a tremendous rise of 7.1 and 9.4 times the normal, respectively (Figure, D).

Table I. The effect of 5 IU of insulin on blood glucose of *O. striatus*

Time of insulin injection	Blood glucose level, mg%
0	107.7
0.5	82.25
1.0	77.0
1.5	47.75
2.0	70.25
2.5	80.50
3.0	110.75

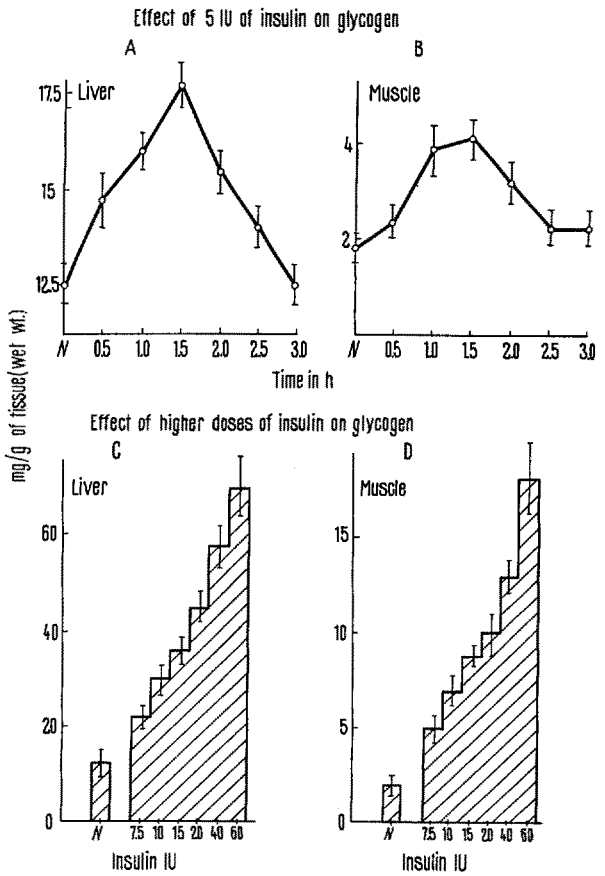

Table II. The effect of higher doses of insulin to *O. striatus*

Dose of insulin injection in IU	Minimum blood glucose reached in mg%
7.5	44.67
10.0	41.00
15.0	32.00
20.0	28.33
40.0	21.33
60.0	15.00

[1] B. SESHADRI, Curr. Sci. *28*, 121 (1959).
[2] B. SESHADRI, Proc. Indian Acad. Sci. *49*, 264 (1959).
[3] B. SESHADRI, J. scient. ind. Res. *20C*, 299 (1961).
[4] B. SESHADRI, J. Anim. Morph. Physiol. *11*, 302 (1964).
[5] R. W. ROOT, F. C. HALL and I. E. GRAY, J. biol. Chem. *91*, 27 (1931).
[6] A. KEMP and A. J. M. KITS, Biochem. J. *56*, 646 (1954).

The results of the present study reveal that the glycogen content of both liver and muscle rises above the normal level concomitent with the occurrence of hypoglycaemia noticed earlier[4]. Compared to the liver, muscle possess a very small content of glycogen. The glycogen content of liver is more than 6 times the normal level in muscle. On the contrary, the rise is more pronounced in muscle as can be seen from the Figure, C and D, following the administration of any dose.

Following islectomy, the fish liver glycogen content was observed to fall[7]. On the contrary, massive doses of insulin injections of 40 IU were found to raise the muscle glycogen but not that of liver of certain marine fish[5]. Glycogen was found to reduce under those conditions.

It can be seen from Tables I and II that there is a concomitant fall in the blood glucose level with the corresponding rise in the glycogen content of the liver and muscle (Figure, A–D) at the various doses of insulin injected. The disappeared glucose might have been converted into glycogen in these tissues due to the injected insulin. Such a possibility of direct action of insulin on the liver and muscle, where glycogen is stored, has been demonstrated very clearly in mammals[8–12]. Further recent studies have also shown that the rise in glycogen could be due to the fact that insulin can inhibit the release of glucose from the liver[13]. Both increased acceleration of glycogen synthesis in these tissues and an inhibition of release of glucose could account for the high glycogen content in these tissues of *O. striatus*.

The rise of glycogen in the liver is not as fast as that observed for muscle, though the muscle glycogen level always remained far below that of liver. It is worth pointing out here that, though the normal liver glycogen was six times that of muscle, it was reduced to less than 4 times following heavy doses of insulin injection. This can be attributed to the very low glycogen present in the muscle at the beginning whose rate of increase has become more pronounced following insulin administration.

The results obtained by ROOT et al.[5] as pointed out earlier, are contradictory to the present observations. They, however, had remarked that their conclusions derived from the study of insulin action were very conflicting and they cannot be in agreement with all other investigations. On the other hand MCCORMIC and MACLEOD[7] showed that insulin was essential for the glycogen synthesis in liver and their results, though indirectly, seem to be in agreement with the present study[14].

Résumé. Des doses variées d'insuline administrées à l'*Ophicephalus striatus* ont augmenté la quantité de glycogène du foie et des muscles. Dans le foie, les doses étant fortes (40 IU et 60 IU), la quantité normale de glycogène a presque quadruplé et elle a plus que septuplé dans les muscles. Le mécanisme possible présidant à l'action de l'insuline est aussi discuté.

B. SESHADRI[15]

Department of Zoology, University of Udaipur (Rajasthan, India), 10th March 1967.

[7] N. A. MCCORMIC and J. J. R. MACLEOD, Proc. R. Soc. *98*, 1 (1925).
[8] J. P. BOUKAERT and C. DE DUVE, Physiol. Rev. *27*, 39 (1947).
[9] C. DE DUVE, Ciba Fdn Colloq. Endocr. *9*, 203 (1956).
[10] C. DE DUVE and H. G. HERS, A. Rev. Biochem. *26*, 149 (1957).
[11] J. P. BERTHET, P. JACQUES, H. G. HERS and C. DE DUVE, Biochem. biophys. Acta *20*, 190 (1956).
[12] P. J. SNYDER and C. F. CAHILL, Am. J. Physiol. *209*, 616 (1963).
[13] G. E. MORTIMORE, Am. J. Physiol. *204*, 699 (1963).
[14] The author expresses his thanks to Prof. R. V. SESHAIYA, Director, Marine Biological Station, Portonovo, India, for the help and encouragement shown in this work.
[15] Present address: Dept. Ob-Gyn. Biochem., K. U. Med. Center, Kansas City, Kansas 66103, USA.

Serum Desoxy-Ribose Compounds in Experimental Myocardial Infarction

Myocardial infarction provides a unique opportunity to study biologic response to a relatively limited injury, in which certain changes are the consequence of a general biologic response rather than of the pathology, at least in the acute stage. Leakage of certain enzymes from the myocardium into the blood stream is already known under such circumstances. LYSENKO[1] was the first to notice DNA decomposition products in the serum of patients with myocardial infarction and angina pectoris. No other data dealing with observations based either on animal experiments or on patients have appeared in literature. The present investigation is a preliminary report of the work, and describes the experimental results obtained in dogs with experimental myocardial infarction at various time intervals after the operation.

Dogs weighing 10–15 kg were used. Myocardial infarction was produced by two stage coronary ligation following the technique of HARRIS[2]. DNA products were estimated by the method of Diphenyl amine reaction (DISHE[3]) for acid soluble ($HClO_4$ 5%) desoxyribose compounds and acid insoluble desoxy-ribose compounds. Blanks and standard solutions were used following the method of LYSENKO[1]. The investigations were made in 10 normal dogs and in the same dogs after experimental myocardial infarction. The estimations were continued only in those dogs which showed 90–100% ectopic beats 20–24 h after the ligation of the coronary artery.

The acid soluble and acid insoluble desoxy-ribose compounds in the serum of normal healthy dogs varied from 9–24 mg% and 42–77 mg% respectively with the mean values of 15 and 59 mg% (Figure). In dogs with myocardial infarction determinations were made at various intervals after the operation up to the fourth week as shown in the Figure. The concentration of these acid soluble compounds remained high for quite long and was significantly elevated up to the fourth week after the operation. Values not changing much up to 4 h, ranged

[1] L. T. LYSENKO, Fedn Proc. Fedn Am. Socs exp. Biol. *23*, 587 (1964).
[2] A. S. HARRIS, Circulation *1*, 318 (1950).
[3] W. DISHE, in *The Nucleic Acids* (Ed. E. CHARGAFF and J. N. DAVIDSON; Academic Press Inc., New York 1955), vol. 1, p. 287.