TABLE I. Effect of Acetylcholinesterase on Blood of Rats. 200 units given daily to each experimental rat.

Group	Time of blood sampling	Reticulocytes, %	Erythrocytes in millions/mm³	Hematocrit, % cells
Exp. (7 rats)	Before start of inj.	1.2 (.8-1.7)*	7.90 (7.40-8.50)*	45.7
Control (")	Before start of exp.	1.1 (.6-2.2)	8.25 (7.50-8.75)	46.7
Exp.	After 5-7 daily inj.	6.1 (3.4-9.5)	8.15 (7.65-8.77)	45.9
Control	Untreated	1.2 (.8-1.8)	8.18 (7.51-8.55)	46.2
Exp.	After 12-16 daily inj.	2.3 (1.2-4.6)	9.30 (9.11-9.44)	46.3
Control	Untreated	1.3 (.8-2.0)	8.07 (7.76-8.78)	45.9

* Range given in parentheses.

Discussion. Acetylcholinesterase administration caused a marked increase in rate of formation or release of reticulocytes and erythrocytes. The fact that hematocrit values were not significantly changed, in spite of an increase of erythrocyte numbers, indicates that the Acetylcholinesterase causes an increased production of cells that are smaller than average in volume. Our present data do not indicate whether or not cholinesterase activity bears any relationship to plasma erythropoietic stimulating factor, although such a possibility is not precluded. Experiments are being continued in the effort to clarify this question.

Summary. Daily subcutaneous injections of 200 units Acetylcholinesterase into rats caused stimulation of erythropoiesis demonstrated by significant reticulocytosis within 7 days and increased erythrocyte counts after 14 days. Hematocrit values did not change significantly.

1. Davis, J. E., *Am. J. Physiol.*, 1946, v147, 404.
2. ———, *Fed. Proc.*, 1960, v19, 67.
3. Goldwasser, E., Jacobson, L. O., Fried, W., Plzak, L., *Science,* 1957, v125, 1085.
4. Davis, J. E., *Fed. Proc.*, 1958, v17, 33.
5. Gordon, A. S., *Physiol. Rev.*, 1959, v39, 1.

Received June 14, 1960. P.S.E.B.M., 1960, v104.

Effects of Acute Hypoglycemia on Rat Testis. (25957)

R. E. MANCINE, J. C. PENHOS, I. A. IZQUIERDO AND J. J. HEINRICH
(Introduced by R. Hertz)
Inst. de Anat. General y Embriol. Fac. de Ciencias Med., and Inst. de Med. y Biol. Exp., Buenos Aires, Arg.

Hypoglycemia is known to exert a deleterious effect on certain endocrine glands and on cerebral structures(2,3,4) but no effect has been reported on male gonads. As these are known to consume glucose mainly(1), we attempted this histological study of the testis of rats submitted to acute hypoglycemia.

Material and methods. 318 male albino rats were used. They were separated as follows: A) Animals in which one hypoglycemic coma was induced: *Group 1*: 62 prepubertal rats weighing 35-50 g; 28 received 200 or 400 I.U. of regular insulin/kg, and 34 received Tolbutamide (2 or 3 g/kg). Insulin was injected subcutaneously and tolbutamide was given by gastric tube. Animals were killed or died in coma. *Group 2*: 84 adult rats weighing 180 to 440 g; 40 received insulin and 44 tolbutamide, at same doses and by same routes as in Group 1, and also killed or died in coma. *Group 3*: 26 adult rats weighing 200-400 g; 16 received Insulin and 10 received tolbutamide as in preceding groups, but these were injected intraperitoneally with 10 ml/kg of 10% glucose several times after administration of the hypoglycemic drug; these animals did not die in coma but were killed 5-8 hours after injection of hypoglycemic agent. B) Animals in which 2 to 5 hypoglycemic comas were induced. *Group 4*: 62

adult rats in which 2, 3, 4 or 5 insulinic comas were induced by same procedure, at intervals of 4 days between comas. Animals withdrawn from each coma by administration of 10 ml kg 30% glucose given by gastric tube; 20 animals were killed or died during second, third, fourth or fifth coma, and 42 were sacrificed 1, 7, 18 and 34 days after recovery from each coma. C) Control groups: *Group 5*: 44 adult and 24 prepubertal rats which received saline solution by any route used for injection of insulin, tolbutamide or glucose. *Group 6*: 16 rats submitted to experimental conditions related to those suffered by groups 1 to 4, *i.e.*, 5 to 10 I.U. of ACTH, metrazolic convulsions (4 to 6 g), and barbituric comas (60 to 80 mg/kg).

All rats were fasted at least 12 hours before administration of hypoglycemic drugs. Glycemia was controlled in all groups by classical Somogyi-Nelson method. Testes were removed immediately after death of animal, and cut in slices not more than 1 mm wide. These slices were placed in Bouin, Zenker or Helly's fluids or in 10% formalin for fixation. For standard histological study 5 μ sections were colored with haematoxylin-eosin, and the following histochemical technics were also used: P.A.S. alsian blue and toluidin blue, (0.200 mg %, at pH 3.5.) Previous incubation with ptialin, hyaluronidase and methanol-chloroform was done as control for presence of substances to be detected by each of aforementioned histochemical stains, *i.e.*, glycogen, mucopolysaccharides and glycoproteins. Also Sudan IV was applied to study lipids.

Results. Rats in Groups 2 and 4 presented injuries, (72% and 100% respectively), in germinal epithelium, and congestion and oedema in interstitial spaces. No damage (0%) was seen in testis of prepubertal animals (Group 1) nor in control Groups 5 and 6; in this latter group only slight congestions were detected. Rats in Group 3, protected with glucose, which showed minimal degrees of hypoglycemia, also showed minimal (20%) or no injuries in their gonads.

Lesions of germinal epithelium consisted of: 1) sloughing, presumably originated in intercellular vacuoles (Fig. 3, 4, 5); 2) intracellular vacuolization of Sertoli cells, spermatogonia and spermatocytes (Fig. 3, 4); 3) Pyknosis of spermatogonia, spermatocytes, and, occasionally of spermatids (Fig. 6, 8); 4) Multinucleated masses of spermatocytes or spermatids (Fig. 6, 7).

Lesions 1 and 2 are present during and after first and second coma while Lesions 3 and 4 appeared in third, fourth and fifth coma. All these cellular alterations increased in number and intensity with repetition of comas. Not all types of lesions were present in each animal; some showed only one or 2 types of injury. The central tubes in a transverse section of glands were always those which showed more damage, and interstitial congestion and oedema were also more intense in this central zone. No lesions were observed in fibroblasts or in interstitial Leydig cells.

Rats which died with lowest levels of glycemia (under 30 mg %) bore more intense injury to their gonads. Group 3, only those animals in which level of glycemia was not completely protected by glucose showed some lesions.

The rats from Group 4 killed at intervals from 1 to 34 days after last coma did not show complete recovery of testicular damage. Desquamative lesions disappeared in animals sacrificed between 7 and 18 days after second or third coma, but nuclear alterations and cellular vacuolization persisted 34 days after fourth and fifth coma.

Histochemical technics showed negative results in all lesions studied. Intracellular and intercellular vacuoles did not show presence of glycogen, mucopolysaccharides, glycoproteins or lipids.

Discussion. Testicular injuries here described resemble description of encephalic injuries produced by hypoglycemia(2,3,4), also consisting of cellular vacuolization and pyknosis. In this sense the brain consumes glucose almost exclusively(1).

That lesions are more intense in animals with lowest glycemias, and that glucose could prevent very low glycemic levels, suggest that hypoglycemia is the specific factor responsible for them. Stress provoked by metabolic disturbance can be disregarded as a causative factor, because administration of ACTH did not produce detectable testicular damage in

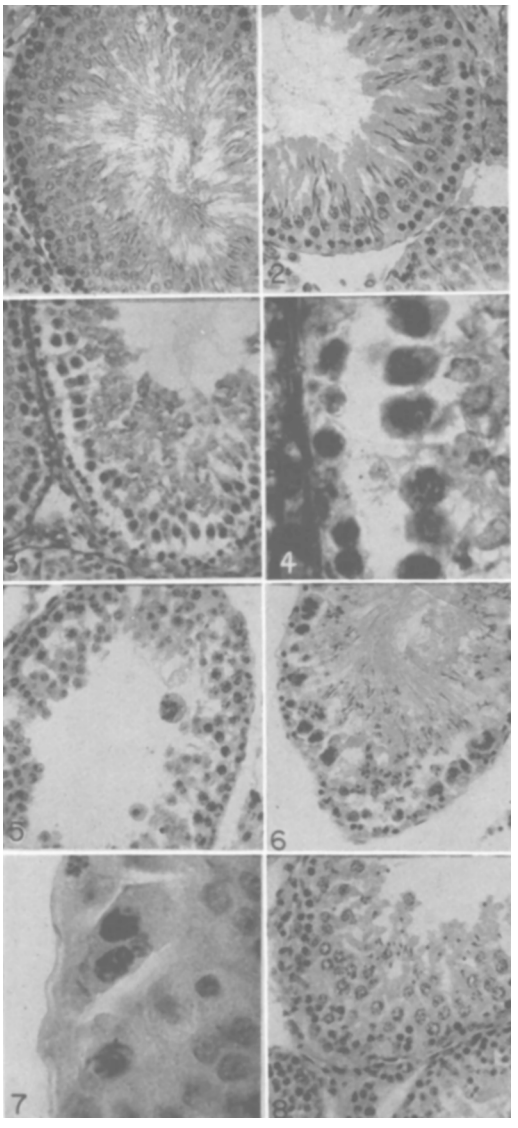

FIG. 1 and 2. Normal seminiferous tubules of adult rats in different stages of germinal epithelium. × 140 and 160 respectively.

FIG. 3. Seminiferous tubule of a rat which died in tolbutamide hypoglycemia. Note vacuolization and sloughing of germinal cells. × 140.

FIG. 4. Same case as Fig. 3. The higher magnification shows vacuoles in Sertoli cells and sloughing of spermatocytes. × 600.

FIG. 5. Seminiferous tubule of a rat killed during first coma. Sloughing has eliminated the layers of spermatids. Multinucleated masses being sloughed off. Intercellular vacuolization is intense. × 160.

FIG. 6. Tubule of rat which died in fifth coma. Note multinucleated cells and other nuclear abnormalities. × 140.

FIG. 7. Multinucleated masses in a tubule from a rat sacrificed at fourth coma. × 600.

animals of Group 6. Other authors proved that high doses of ACTH or corticoids in acute or chronic experiments did not show appreciable changes in the testis(5,6). The possibility that coma or convulsions were nonspecific factors causing testicular damage was also discarded; coma produced by Pentobarbital, and convulsions produced by Metrazol caused only slight interstitial oedema in testis of rats.

Tissue specificity of lesions seems high. No damage in cells, except interstitial congestion, was seen in liver or kidneys of rats bearing serious gonadal injury. This is also backed by lack of lesions in fibroblasts and Leydig cells in intertubular spaces. Injuries do not appear to be easily reversible; their intensity and persistence increased and were related to repetition of comas, and recovery is not complete, several weeks after last coma.

It is apparent that proliferation, differentiation, and greater metabolic turnover of germinal epithelium in adult animals as compared with prepubertal ones, renders them more liable to the deleterious effect of acute hypoglycemia.

Regarding histochemical nature of injuries, as vacuoles were negative to stains used, they may be temporarily ascribed to hydropic imbibition. The sequence of lesions revealed that these vacuolizations appear first and could be responsible for cellular sloughing. Nuclear abnormalities develop later and suggest severe disturbance in metabolism of nucleic acids and cell division. The significance of lack of glucose, which should deprive germinal cells of an important source of metabolic energy, is emphasized by our recent studies (unpublished) on oxygen consumption of rat testis using substrate with different carbohydrates. Also, it was shown that glucose is necessary to maintain male embryonic gonads incubated *in vitro*(7).

Whether injuries are secondary to interstitial oedema, we can only state that the latter was seen alone, with no accompanying tubular damage, but the reverse was not true.

FIG. 8. Rat killed during fifth coma. Injuries here are different as compared with Fig. 6. Pyknosis of nearly all spermatids is the main lesion here. × 160.

Summary. The histology of testis in albino rats submitted to hypoglycemic comas was studied. Comas were produced by either insulin or tolbutamide. No changes were seen in prepubertal animals, but in adults, injuries were seen in the seminiferous epithelium, coning of cellular and intercellular vacuoles, sloughing, nuclear pyknosis and multinucleated cells. Congestion and oedema were invariably present in the interstitium, though no injury was seen in any other testicular structure. Lesions increased in number and intensity with repetition of comas and recovery after comas was incomplete. Hypoglycemia is considered the specific cause of the injuries.

1. Elliot, K. A. C., Page, I. M., Quastel, J. H., Thomas, Ch., Brain tissue respiration and glycolysis. *Neurochemistry,* Springfield, Ill., 1955.

2. Yannett, A., *Arch. Neurol. Psychiat.,* 1939, v42, 237.

3. Soskin, S., *ibid.,* 1939, v42, 563.

4. Zimmerman, H. M., *Arch. Path.,* 1939, v28, 276.

5. Courrier, R., Baclesse, M., Marcis, M., *J. Physiol.,* 1953, v43, 327.

6. Arvay, A., Balazzy, L., *Acta Physiol. Acad. Sci. Hung.,* 1958, v14, 317.

7. Raty Stenger-Haffen. *Arch. d'Anat. Microsc. et Morphol. Exp.,* 1957, v46, 522.

Received June 16, 1960. P.S.E.B.M., 1960, v104.

Carbon Dioxide Fixation by Actinomyces . . . A Correction. (25958)

LEO PINE

Dept. of Microbiol., Duke University Medical School, Durham, N. C.

Previously(1) I reported results of experiments on carbon dioxide fixation by strains of *Actinomyces* and *Lactobacillus* in which the data indicated a high incorporation of carbon dioxide into the methylene carbons of succinic acid. The data were supported by isolation of beta-alanine formed from succinic acid by the Schmidt degradation done at 40 to 70°C. Recently studies in this laboratory were initiated to determine the mechanism of this reaction in strains of *Actinomyces.* Although the initial experiments reproduced the previous results, isolation of the methylene carbons of succinic acid as ethylenediamine was preferred since it would eliminate several chromatographic steps and simplify the procedure. Although I had been unsuccessful previously in obtaining ethylenediamine by the procedure of Phares and Long(2) the use of a better grade fuming sulfuric acid and high temperatures permitted adequate formation of ethylenediamine. With some dismay, it was found that the carbon dioxide formed from the ethylenediamine was totally inactive. Subsequently, it was found that failure to adequately trap sulfur dioxide fumes formed by the reaction(3) accounted for the reduced specific activity in the carboxyl carbons. However, the close correlation of the specific activity of the isolated beta-alanine with that predicted is unexplained. The present results clearly show a net fixation of CO_2 into the carboxyl carbons of succinic acid. The remaining data published regarding fixation and formation of CO_2 are valid. It is, therefore, concluded that anaerobic fixation of CO_2 into succinic acid by *Actinomyces* primarily occurs *via* a Wood-Werkman reaction. In air, the oxidation of some intermediate of glucose metabolism results in formation of CO_2 and consequently reduces the specific activity found in the succinic acid.

I regret the error in the analyses and the concomitant circumstances which lead to a misinterpretation of the data. I would like to apologize for any inconvenience the use of such data may have caused.

1. Pine, L., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 468.

2. Phares, E. F., Long, M. V., *J. Am. Chem. Soc.,* 1955, v77, 2556.

3. Katz, J., Abraham, S., Chaikoff, I. L., *Anal. Chem.,* 1955, v27, 155.

Received June 21, 1960. P.S.E.B.M., 1960, v104.