Passage of Insulin through the Wall of the Gastro-intestinal Tract of the Infant Mouse

Mosinger, Placer and Koldovsky¹, in a recent communication, have shown that insulin administered orally to suckling rats 2 and 8 days old causes a large drop in blood-sugar levels, but does not do so in 21- and 30-day old rats. Previously I had found that the gut of the 8-day old mouse is permeable to insulin but that this permeability declines thereafter. This work was quoted by Halliday² and, although Mosinger *et al.* refer to that paper, they do not mention my findings.

LAB grey mice were used, the young being removed from the mother 2 hr. before the administration of insulin and kept in an incubator at 37° C. for the duration of the experiment. Administration was direct to the stomach with a polythene tube on a tuberculin syringe. Injections were made intraperitoneally. The insulin used was standard crystalline insulin (B.D.H., A. and H. 22 units/mgm.) dissolved in 0.9 per cent saline acidified with hydrochloric acid to pH 2.5. Controls were given an equivalent dose of acidified saline. Observation of convulsions and death did not provide a satisfactory method of measuring the passage of the intact insulin molecule through the gut wall, as infant mice survived hypoglycaemia far longer than did adults. This agrees with the findings of Himwich, Fazekas and Hamburger³, who showed that in the rat the capacity of the infant brain to endure hypoglycaemia is associated with its lower cerebral metabolic requirement. Therefore, estimations of serum sugar were undertaken. Repeated bleedings of 8-day old mice necessitated micro-methods of sampling and estimation. Blood samples each of c. 5 μ l. were taken from the tail into capillary tubes before administration of the hormone, and at 30 min. and 60 min. after. The tubes were sealed and stored at 4° C. until tested. The serum was separated by centrifugation and 2- μ l. samples tested in duplicate by the method of Stern and Kirk⁴. An 'Agla' micrometer syringe was used for the titrations.

The results are shown in Fig. 1. It is evident that the majority show a significant fall in serum sugar at 7, 8 and 16 days, and that only a small minority show this at 22 days. At 8 days of age, doses of 1 unit/gm. may be effective. At 22 days even doses of 8-10 units/gm. usually have no effect. It is evident

from these results that the gut of the suckling mouse at 8 days, and even at 16 days, is permeable to insulin administered orally in very large doses, and that this permeability has almost disappeared at 22 days. The disappearance of permeability to insulin appears to occur rather later and to be less clear-cut than that to antibodies in the suckling mouse. The doses necessary are enormous in comparison with those of the order of 0.001 unit/gm. which are effective by parenteral injection. The wide individual variation in response to the oral administration of insulin at all ages studied is surprising, and for this I have no explanation to offer.

I am indebted to Prof. F. W. R. Brambell and Mr. W. A. Hemmings for their interest and advice during the course of this work.

W. A. KELLY

Department of Zoology,
University College of North Wales,
Bangor.
Jan. 23.

¹ Mosinger, B., Placer, Z., and Koldovsky, O., *Nature*, **184**, 1245 (1959).

² Halliday, R., *J. Endocrinol.*, **18**, 56 (1959).

³ Himwich, H. E., Fazekas, J. F., and Hamburger, E., *Endocrinol.*, **33**, 96 (1943).

⁴ Kirk, P. L., "Quantitative Ultramicroanalysis" (Wiley: Chapman and Hall, 1950).

Seasonal Differences in Ovulating Hormone in the Merino Ewe

CESSATION of cyclic ovarian activity in ewes during anoestrus is thought to be associated with changes in the level and/or type of gonadotrophic substances reaching the ovary¹. This supposition cannot be tested by direct means at the present time, but a recently developed technique for synchronization of ovulation in ewes² suggests possibilities of an indirect examination.

Merino ewes were injected daily with 10 mgm. of progesterone in oil intramuscularly for 14 days in either April (the middle of the breeding season) or October (anoestrus). At the same time as the final progesterone injection each ewe received 500 I.U. of pregnant mare serum gonadotrophin subcutaneously. 48 hr. later groups of ewes were injected with either 300 I.U. of human chorionic gonadotrophin intra-

Fig. 1. Values of serum sugar in mgm./100 ml. 60 min. after oral administration of insulin to young mice of 7, 8, 16 and 22 days of age according to dose in units/gm. The corresponding approximate scales for blood sugar are inserted on the right for comparison with the serum sugar scale on the left: the blood sugar values being derived from the haematocrit values of 33 per cent for 8-day and 42 per cent for 16- and 22-day-old mice. The solid horizontal line represents the mean serum sugar-level before the administration of insulin and the broken line the value below which individual experimental results may be considered significant at the 5 per cent level.