The Digestive Capacity of Rat Yolk-Sac in Organ Culture

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Following Goldman's (1909) original demonstration of intense phagocytic activity in the rat yolk-sac, recent work has been concerned with the functional significance of the endocytosis manifest by the cells of this organ (Al-Abass & Schultz, 1966; Lambson, 1966; Schultz, Reger & Schultz, 1966; Krzyzowska-Gruca & Schiebler, 1967; Lloyd, Beck, Griffiths & Parry, 1968; Beck & Lloyd, 1968). Beck & Lloyd (1968) have suggested a role in embryotrophic nutrition, whereas Wilson, Beaudoin & Free (1959) have postulated a function primarily designed to protect the embryo from contact with noxious macromolecules. In either case intralysosomal digestion of endocytosed material would be important. Previous studies *in vivo* and *in vitro* (summarized by Beck & Lloyd 1968) used horseradish peroxidase as marker and demonstrated a progressive fall in enzyme activity in rat yolk-sac after uptake of this enzyme.

In the present work ¹²⁵I-labelled bovine serum albumin was prepared (McConaghey & Dixon, 1966), freed from inorganic iodide and denatured with acetic acid. Inbred Wistar rats (200–225 g.), when 17.5 days pregnant, were injected intravenously with albumin solution (0.75 mg./kg. body wt.) in saline. Serial removal of conceptuses enabled the uptake of albumin by the yolk-sac to be estimated. Activity, expressed as c.p.m./mg. of yolk-sac protein, reached a peak after 2–5 hr.

Villous portions of albumin-containing yolk-sacs were grown on tantalum grids with 5.0 ml. of medium TC 199 (Trowell, 1954; modified by Parry, Beck & Lloyd, 1968); control explants were deep-frozen. At intervals of up to 3 hr. 1 ml. portions of the medium were withdrawn and replaced with

fresh medium. The trichloroacetic acid-soluble and -insoluble radioactivity was measured in each sample of medium and thus the total radioactivity released over a 3 hr. incubation computed. The acid-soluble and acid-insoluble radioactivity in unincubated and post-incubation explants was also measured. The experiments show a rapid conversion of acid-insoluble into acid-soluble material during incubation, indicating that the ingested albumin was subject to hydrolysis. Most of the acid-soluble material was released into the medium. Although cytologically and histochemically inapplicable, a great advantage of this method over the use of peroxidase as marker is that it can monitor the appearance of breakdown products from protein hydrolysis. The method is thus potentially capable of determining the comparative rates of hydrolysis of different radioactively labelled substrates and the effects of endocytosed materials on lysosomal catabolism.

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