The Effect of Cycloheximide on Tissue Clearing-Factor Lipase Activity

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(*Received* 8 *June* 1967)

The clearing-factor lipase activity of adipose tissue from fed rats has been shown to fall rapidly on starvation (Hollenberg, 1959; Cherkes & Gordon, 1959; Robinson, 1960; Páv & Wenkeová, 1960; Wing & Robinson, 1967). A similar rapid loss of enzyme activity follows the injection of puromycin (Wing, Salaman & Robinson, 1966). These results indicate that under such conditions the enzyme in adipose tissue can be rapidly degraded or converted into an inactive form (see Wing & Robinson, 1967).

In an extension of the work on tissue clearing-factor lipase, evidence was obtained that the injection of puromycin also caused the activity of the enzyme in heart, lung and diaphragm to fall rapidly (C. J. Fielding, unpublished work). Because of the expense of using puromycin in experiments *in vivo* on a large scale, cycloheximide, which has been shown to produce rapid and virtually complete inhibition of protein synthesis in mammalian tissues (Young, Robinson & Sacktor, 1963; Trakatellis, Montjar & Axelrod, 1965; Warner, Girard, Lathan & Darnell, 1966), has been used in a continuation of these studies, the results of which are now reported.

Cycloheximide, 3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide [Sigma Chemical (London) Co. Ltd., London, S.W. 6], was injected intraperitoneally in 0·9% NaCl solution into male rats (120–140g. body wt.) of the Wistar strain. The dose given (5mg.) was similar to that which has been shown to cause greater than 90% inhibition of amino acid incorporation into protein in a variety of mammalian tissues (Young *et al.* 1963; Trakatellis *et al.* 1965; Warner *et al.* 1966). The animals were either given a laboratory stock diet *ad lib.* or starved for 16hr. The injections were made with the animals under light ether anaesthesia and were carried out between 8a.m. and 9a.m. Injections of 0·9% NaCl solution alone had no significant effect on tissue clearing-factor lipase activity.

At intervals after the injection of cycloheximide, groups of animals were killed under ether anaesthesia, and the epididymal fat bodies, diaphragm, heart and lungs were removed, rinsed in 0·9% NaCl solution, dried and weighed and stored at 0°. When all the animals in a group had been killed, acetone–ether-dried preparations were made from the grouped organs and the clearing-factor lipase activity of homogenates of these preparations was determined. The methods used were essentially as described previously (Robinson, 1963; Wing *et al.* 1966). Clearing-factor lipase activities are expressed as μmoles of free fatty acids released from chyle triglycerides/hr./epididymal fat body, heart, lung or diaphragm. Expression of the results in terms of tissue wet weight does not affect the findings significantly.

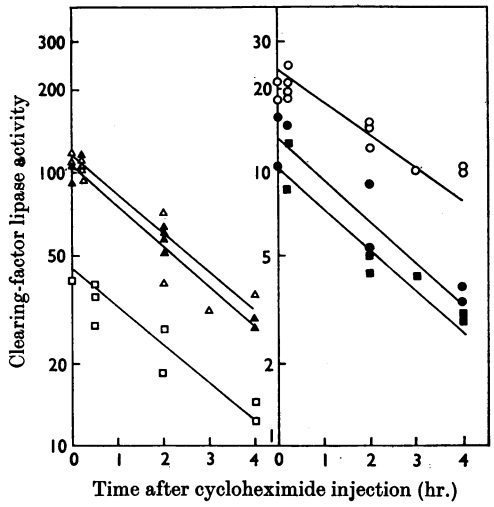

Fig. 1. Effect of cycloheximide injection on tissue clearing-factor lipase activity. Groups of rats were injected with 5mg. of cycloheximide in 0·9% NaCl soln. intraperitoneally. The rats were either given their normal diet *ad lib.* or starved for 16hr. All injections were carried out between 8a.m. and 9a.m. At intervals the rats in each group were anaesthetized and various tissues were removed for clearing-factor lipase assay. Each point represents a determination made on tissue from at least four animals. Enzyme activities are expressed as μmoles of free fatty acids released/hr./organ. Fed rats: △, heart; □, epididymal fat body; ○, diaphragm; ■, lung. Starved rats: ▲, heart; ●, diaphragm.

The results in Fig. 1 show that, after the injection of cycloheximide, clearing-factor lipase activity falls rapidly in all the tissues examined. Over the time-period studied, the decline in activity appears to be exponential with a half-life of about 2hr. The

rate of fall of activity in heart and in diaphragm is similar in fed rats and in rats that have been starved for 16hr. Similar rates of loss of activity have also been observed in lung tissue from fed and starved rats. In adipose tissue, the enzyme activity after starvation for 16hr. has already fallen to about 10% of that in the fed animal (Wing & Robinson, 1967) and in such animals it does not decline further after cycloheximide injection.

It is evident from Fig. 1 that tissue enzyme activities frequently varied considerably from one experiment to another, despite the fact that each value was derived from an assay of the grouped tissues of at least four animals. The reason for this variation is not understood.

The finding that the clearing-factor lipase activity of heart, diaphragm and lung from fed and starved rats and of adipose tissue from fed animals is lost so quickly after cycloheximide injection is most readily explained on the assumption that in such tissues most of the enzyme, or an essential cofactor or activator thereof, is normally being synthesized and degraded at a rapid rate. However, the possibility that cycloheximide may cause the conversion of the enzyme into an inactive form, without enzyme degradation, cannot be entirely excluded (Wing & Robinson, 1967).

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Biochem. J. (1967) **104**, 46c

Quantitative Determination of Free and Conjugated Bilirubin by Diazo Coupling and a Liquid-Extraction and Column-Chromatographic Technique

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(*Received* 19 *June* 1967)

Clarification of the relationship between direct-reacting and indirect-reacting bilirubin fractions of the van den Bergh test as constituting bilirubin and bilirubin glucuronide (Billing, 1954; Schmid, 1956, 1957; Talafant, 1956; Billing, Cole & Lathe, 1957) has led to the advent of several techniques for the partitioning of the bilirubin pigments. The reversed-phase partition column-chromatographic technique originally described by Cole & Lathe (1953) utilizing silicone-treated kieselguhr (Howard & Martin, 1950) appeared to be one of the most promising ways to separate bilirubin and its glucuronide conjugate. Although the extraction method used by Schmid (1956, 1957) served its purpose well, it was not quantitative (R. Schmid, personal communication). The purpose of the work now reported was to modify these latter methods to accomplish the dual purpose of a quantitative extraction and a chromatographic separation of bilirubin and its glucuronide on a routine basis. In addition, a commercially available column support has now obviated the laborious task of silicone treatment of kieselguhr used as the original chromatography support.

A 3ml. sample of plasma or of tissue homogenate is transferred to a glass-stoppered centrifuge tube and 4·5ml. of 95% (v/v) ethanol and 3ml. of a concentrated diazo reagent (Weber & Schalm, 1962) are added. The stoppered tube is then shaken moderately for 1hr. Then 9ml. of water, 9·0ml. of butan-1-ol and 1·25g. of crystalline solid Na_2SO_4 are added. The stoppered tube is then shaken vigorously for 15min. After the shaking, the tubes are centrifuged at 1300*g* for 5min. The butanol layer is transferred to a round-bottomed evaporation flask. The layer remaining in the tube plus the protein precipitate is then repeatedly extracted with 2ml. portions of fresh butanol until azo-pigment is no longer extracted into the butanol. The contents