By expressing the glycolysis through the velocity constant corrected for the hematocrit, the variations due to the concentration of glucose are automatically eliminated. However, it has to be kept in mind that glycolysis is not a simple process but consists of a chain of interrelated reactions and therefore the use of a single velocity constant may be an oversimplification which is not free from reasonable criticism.

Whereas the relationship between initial glucose level and glycolysis is strongly suggestive of a first order reaction, the disappearance of the glucose followed at shorter intervals during the first 2 h approximates to a straight line more consistent with a reaction of order zero. We have no satisfactory explanation for this discrepancy.

Since under normal circumstances the range of the sugar levels in the blood is relatively small, no significant error can be made if one expresses the glycolysis in the conventional way. However, this method becomes unreliable when the glycolysis is followed during spontaneous or induced sugar variations.

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Riassunto

Studiando la glicolisi di globuli rossi umani è stata notata una correlazione statisticamente significativa tra livello iniziale di glucosio e glicolisi, espressa in mg di glucosio utilizzati da 100 ml di globuli rossi per ora. In condizioni di carico sperimentale con glucosio la correlazione ha avuto un comportamento variabile.

Activation of Cathepsin in Fatty Liver

It has been shown in recent years that cathepsin activity of liver cells is mainly located within mitochondria[1]. De Duve *et al.*[2] have found that the cathepsin which is active on haemoglobin is bound to a special type of particles, which have the same sedimentation characteristics such as light mitochondria. These particles have been called *lysosomes*. They contain practically all acid phosphatase, β-glucuronidase, ribonuclease, desoxyribonuclease and cathepsin activities of the liver cells. The activity of these enzymes is very low when the lysosomes are intact, but increase many times as a result of damaging treatments. These also produce the displacement of the bound enzymes from the particles to the suspension fluid. Many different treatments produce similar effects: homogenization in Waring blendor, suspension in hypotonic solutions, incubation at 37°C for a short time, addition of salts, addition of substances which decrease the surface tension.

The presence of mitochondrial damage in fatty livers has been described[3]. Morphological modifications of mitochondria consist mainly in the swelling of the particles. Swollen mitochondria show higher permeabilities to many substances originally contained inside their body, as ribonucleic acid[4], cytochrome *c*[5], pyridine nucleotides[6], adenosine phosphates[7], thiamine pyrophosphate[8], citrate[9]. These substances are displaced from the mitochondrial body to the suspension fluid as a consequence of treatments which produce mitochondrial swelling. These treatments resemble strongly those which produce lysosome damage. It seems then not improbable that lysosome damage exists in fatty livers. Strong increase of acid phosphatase activity of fatty livers has been reported[10]. In these conditions, a redistribution of the enzyme takes place, as a large part of it is displaced from mitochondria into the fluid part of the homogenate. An increase of β-glucuronidase in fatty livers has been reported by Mills *et al.*[11].

Thus it was interesting to study the behaviour of cathepsin, another enzyme present in lysosomes. Albino rats weighing 150–180 g were used. They were fed on a standard diet. Fatty infiltration of the liver was obtained either by 2 subcutaneous injections of carbon tetrachloride (0·2 ml of a 20% solution in olive oil, each day), or also by one subcutaneous injection of white phosphorus (0·1 ml of the 0·5% solution in olive oil). The rats were killed by decapitation 24 h after the last injection. The liver was immediately dissected and transferred to a cold room at 2°C.

10% homogenates were prepared in a Potter-Elvehjem homogenizer, two types of tissue suspensions being prepared in each case: the first one was prepared with 0·25 *M* sucrose, the second one with 0·25 *M* sucrose containing 0·1% *Triton X–100* (obtained through the courtesy of Prof. H. G. K. Westenbrink). This was used to produce maximum activation of cathepsin, as a result of the disintegration of the particles (Wattiaux and De Duve[12]). In order to avoid mechanical damage to the particles in the case of the homogenates prepared with 0·25 *M* sucrose, mitochondria were not isolated and the enzyme determinations were performed on the whole homogenates. These were used within 10 min after the death of the animal.

Cathepsin activity was determined at 37°C by a modification of the method of Anson[13] described by Gianetto and De Duve[14]. The reaction mixture had the following composition: 0·17 *M* acetate buffer, pH 5·0, 0·00026 *M* haemoglobin, the enzyme and 0·25 *M* sucrose to 3 ml. The reaction was stopped by adding 4 ml of ice-cold 0·3 *M* trichloroacetic acid. The mixture was cooled immediately and filtered in the ice box, in order to decrease the hydrolysis of sucrose, the products of which interfer with the

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Cathepsin activity of the liver of normal rats and of rats treated with steatogenic poisons.

The standard deviation is indicated after each average. Fat content of normal liver was mg 40·8 ± 4·7/g wet tissue. Those of the liver of the rats treated with CCl_4 or with phosphorus were respectively mg 71·1 ± 8·6 and 63·0 ± 7·8.

Amount of enzyme used mg	Normal rats		Rats treated with CCl_4		Rats treated with phosphorus	
	μg tyrosine set free in 8 min	μg tyrosine /mg N	μg tyrosine set free in 8 min	μg tyrosine /mg N	μg tyrosine set free in 8 min	μg tyrosine /mg N
Without Triton . . . . 20 .	0	0	7·0 ± 7·0	14·4 ± 14·4	4·6 ± 4·0	9·3 ± 9·0
With Triton . . . . . 20 .	32·7 ± 19·6	57·5 ± 32·3	54·5 ± 19·8	121·7 ± 47·1	58·2 ± 22·6	118·8 ± 50·5
Stimulation % by Triton . .	∞	∞	678·5		116·5	
Without Triton . . . . 50 .	7·3 ± 7·0	5·1 ± 5·0	31·4 ± 13·9	28·3 ± 13·5	26·5 ± 8·8	21·6 ± 7·5
With Triton . . . . . 50 .	62·4 ± 18·3	44·1 ± 16·4	97·7 ± 19·3	78·0 ± 21·2	90·3 ± 23·3	81·2 ± 17·9
Stimulation by Triton . . .	754·8		211·1		240·7	
Without Triton . . . 100 .	14·3 ± 9·6	5·1 ± 3·1	46·2 ± 17·2	20·6 ± 6·2	43·3 ± 20·1	18·1 ± 10·0
With Triton . . . . 100 .	99·7 ± 18·8	34·0 ± 7·8	137·1 ± 48·1	61·6 ± 24·7	122·0 ± 29·2	50·9 ± 16·4
Stimulation% by Triton . .	583·2		196·7		181·7	

colour reaction. All the solutions used, with the exception of trichloroacetic acid, were made either in 0·25 *M* sucrose or in 0·25 *M* sucrose containing 0·1% Triton X–100. Haemoglobin was purified according to GIANETTO and DE DUVE[14]. Three different amounts of enzyme were used for each determination: 20, 50, and 100 mg respectively. For each series 2 samples were prepared: in the first one, the reaction was stopped 2 min after the beginning of the incubation, in the second one it was stopped after 10 min. The aromatic degradation products of haemoglobin were measured on the deproteinized filtrates by means of the Folin-Ciocalteu reagent, with tyrosine as standard. The differences between the readings found at 10 and 2 min were retained as a measure of the enzyme activity. The values are given in the Table as micrograms tyrosine set free in 8 min.

In all homogenates, nitrogen was determined by the usual microkjeldahl technique. Total fat content of each liver was determined by ethereal extraction of the dry powder of the organ in a Soxhlet apparatus.

The Table shows that, in the case of normal liver, practically no enzymatic activity was found in the homogenates prepared without Triton, when the amount of enzyme used corresponded to 20 and to 50 mg of tissue. The activity was present, but very small, only with 100 mg tissue. The addition of Triton to the homogenates produced a strong activation of cathepsin activity. As the values per mg nitrogen were higher with 20 mg tissue than with higher amounts of tissue, it seems probable that in the last case saturation of the enzyme is attained. In the case of fatty livers, the enzyme activity was practically nil with 20 mg tissue, but was evident with 50 mg and was rather high with 100 mg. The addition of Triton produced further stimulation, but this was many times lower than that observed in the case of normal livers. The enzymatic activity in the presence of Triton was higher in the case of fatty liver than in that of normal liver. The difference is quite striking if the values are given per mg nitrogen.

One may conclude from these results that the activity of cathepsin is increased in fatty livers determined by carbon tetrachloride and by white phosphorus. One of the reasons for this increase is probably the damage of lysosomes. This cannot, however, be the only cause for the increase, as it was found also in the experiments made in the presence of Triton X–100.

The morphology of lysosomes has not yet been well established. NOVIKOFF, BEAUFAY, and DE DUVE[15] have succeeded in isolating from rat-liver a lysosome-rich fraction, which mainly consisted of small mitochondria and of a peculiar type of particle, the 'dense body'. 'Dense bodies' have been tentatively identified with lysosomes. The morphology of 'dense bodies' closely resembles that which was described by RHODIN[16] and by ROUILLER and BERNHARD[17] for 'microbodies'. These are present also in normal liver, but increase strongly in fatty livers produced by carbon tetrachloride or also by partial hepatectomy[18]. An increase of mitochondria with a very small diameter, probably to be identified with microbodies, was described in fatty liver by carbon tetrachloride also by DIANZANI and BAHR[19]. These facts may be interesting in order to understand the nature of the increase of cathepsin in fatty liver.

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Riassunto

L'attività cateptica del fegato grasso ottenuto mediante trattamento parenterale con CCl_4 o con fosforo nel ratto è aumentata rispetto al normale. Nel normale, gli omogenati hanno una attività cateptica molto bassa, che aumenta di molte volte se si aggiunge al mezzo il Triton X–100. Nel fegato grasso, invece, si osserva una discreta attività cateptica già in assenza di Triton; questo determina un aumento dell'attività, ma in misura percentualmente minore che nel normale. In presenza di Triton, l'attività cateptica dell'omogenato di fegato grasso è superiore a quella del fegato normale. L'aumento dell'attività enzimatica non è quindi interamente attribuibile alla lesione delle particelle che contengono l'enzima.

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