RUSSELL L. DETER and CHRISTIAN DE DUVE

bodies, is observed in the liver within an hour after injection (see reference 11). It has already been shown by Berthet (6), that this phenomenon

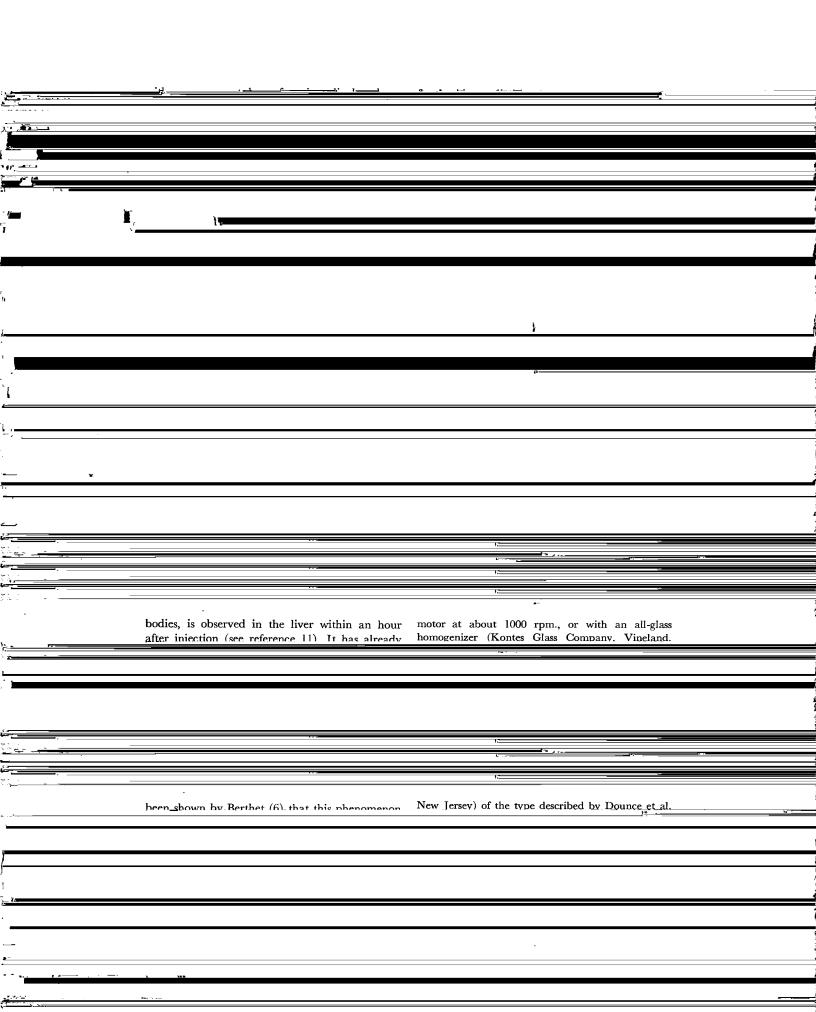motor at about 1000 rpm., or with an all-glass homogenizer (Kontes Glass Company, Vineland, New Jersey) of the type described by Dounce et al.
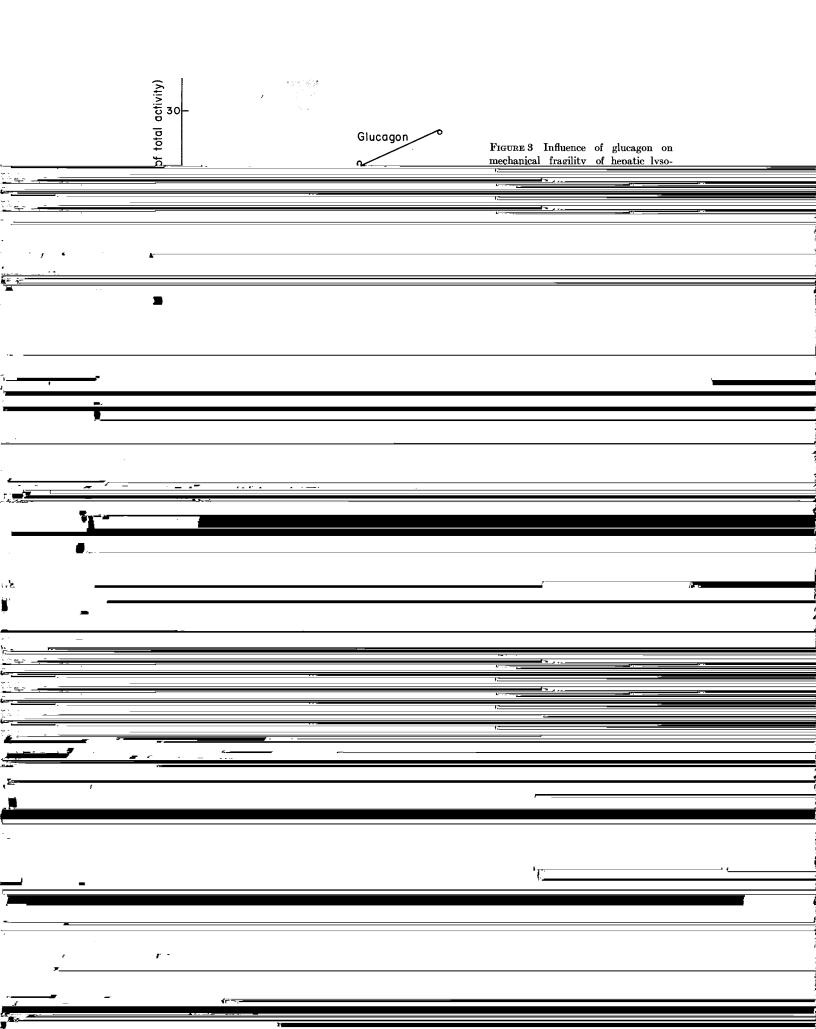
Appelmans and de Duve (1) was followed to assess   et al. (9) and at a temperature of 0–2°. Special care

their osmotic sensitivity. The preparations were di-   was taken to reduce convection artifacts by following

luted to final sucrose concentrations ranging between   the precautions recommended by these authors. A
0.20 and 0.025 M and kept at 0° for 30 min; they were   portion of the extracts used in preparing the gradients

then brought back to the original 0.25 M concentra-   was also centrifuged at high speed for the determina-

tion by addition of a suitable amount of concentrated   tion of unsedimentable activities, as described above.

sucrose.   All the fractions as well as the original extract were

Sedimentation-velocity analysis was carried out on   assayed for various enzymes. The extracts were also

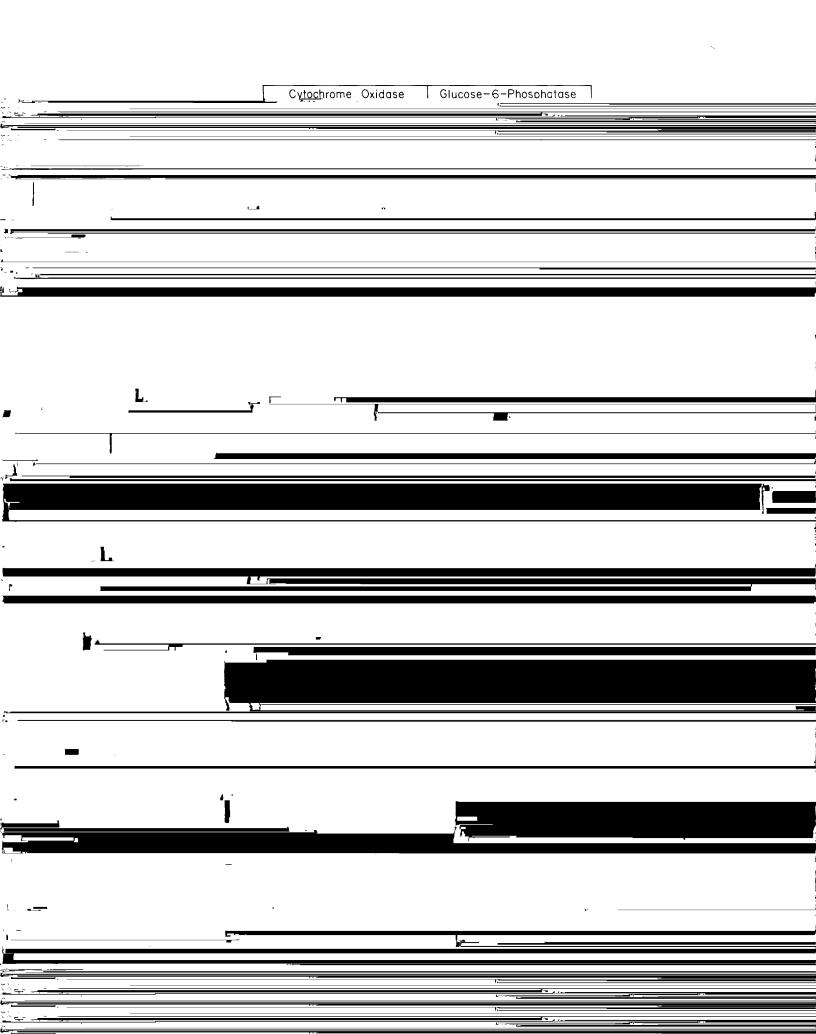cytoplasmic extracts by differential density-gradient   analyzed for free acid phosphatase activity before and

scribed by Beaufay et al. (4). They were further con-

verted to a sedimentation coefficient scale by means

of the approximate formula given by Beaufay et al.

(3), assuming a simple linear relationship between

sedimentation coefficient and radial distance. When

several histograms were combined, their abscissa

scales were divided into 12 equal sections and the

design permitted it, the results were analyzed by

factorial variance analysis and the significance of the

observed effects was estimated from the value of the

variance ratio F. Differences between individual

means were evaluated by the multiple range test of

Student-Newman-Keuls.

FIGURE 3 Influence of glucagon on mechanical fragility of hepatic lyso-

concentration was chosen for a more detailed time study of the phenomenon. Potter homogenates or cytoplasmic extracts were used in these experi-

shock, over and above the amount set free by

Potter homogenization.

solvent. As shown in Table V, the increased

osmotic fragility of the lysosomes induced by

formed on Dounce homogenates from animals

homogenates. There is even an indication that

killed 1½ hr after injection of either glucagon or

more particles are disrupted by the osmotic shock

| Cytochrome Oxidase | Glucose-6-Phosphatase |

tion diagram. The *intermediate component* was esti-   not be ascribed to improper control of the centrif-

mated by difference. Finally, the *median sedimenta-*   ugation conditions, since a similar increase in the

tion coefficient was obtained from the abscissa value   median sedimentation coefficient of cytochrome

corresponding to the point on the boundary curve   oxidase was not observed. A slight, though

result of glucagon administration. In the cases

of cathepsin D and acid deoxyribonuclease.

which are least affected by an increase in the slow

component, the increment in median sedimenta-

tion coefficient is of the order of 50%. If the
particles are spherical and their density and shape

remain unaltered, this would correspond, on an

a boundary in higher centrifugal fields and is at

least 50% latent, suggesting that it is associated

with true lysosomal particles. It is possible that

small lysosomes appear as an outcome of the fusion

processes believed to be involved in autophagic-
vacuole formation, or that primary lysosomes are

formed as a result of glucagon injection, perhaps

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9. DE DUVE, C., J. BERTHET, and H. BEAUFAY. 1959.