The inhibition of net release of TG by the liver from alloxan-diabetic animals may result from an increased oxidation of fatty acids, a decreased rate of esterification of fatty acid to triglyceride, an inhibition of outward transport of TG in the very low-density lipoproteins, or any combination of these factors. This decreased release of TG may also be related to a diminished rate of synthesis of lipoprotein protein. It has been well documented that fatty acid oxidation is increased in livers from diabetic animals.[11] In the experiments reported here, the rapid uptake of NEFA and the inhibition of TG output by the livers from diabetic animals was accompanied by an accelerated rate of formation of ketone bodies (M. Heimberg, unpublished observations). Effects of alloxan diabetes and insulin on TG formation, lipoprotein synthesis, and outward hepatic transport of TG are under investigation.

The nonesterified fatty acid levels in the blood of diabetic animals are higher than in normal animals.[11] A considerable proportion of this supply may be converted to triglyceride even though NEFA oxidation is accelerated. Much of this TG may then be retained within the diabetic liver, giving rise to the fatty liver, while a fraction of the hepatic TG may be released into the blood. It is necessary to note, however, that the livers from normal rats released much more TG than did livers from diabetic animals when the livers were exposed to equivalent amounts of NEFA at low or high concentration of NEFA in the medium.

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## Effect of thalidomide on rat liver regeneration and diaphragm carbohydrate metabolism

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THE implication of thalidomide (DL-N-(2,6-dioxo-3-piperidyl)phthalimide) in human fetal growth and developmental abnormalities such as phocomelia, has led to a variety of researches in mammalian and avian species. Teratogenic effects were described by several workers for the rabbit and mouse, among other species; but resorption to the exclusion of any malformations was observed in the rat, as was also the case with the monkey. DiPaolo[1] pointed out that when the drug was administered to the pregnant mouse before limb bud formation, the toxic effects became quite prominent. The metabolism of thalidomide in the rat has been shown to be rapid[2] and, in contrast to earlier reports, abnormalities in rat fetuses were reported by King and Kendrick.[3]

In the light of the damaging action of thalidomide, especially when administered prior the to advent of the limb buds, it was thought that the agent might elicit a definite effect on the highly proliferative

process of liver regeneration in partially hepatectomized animals, which displays certain similarities to fetal growth. In this conjunction, the writer[4] has shown that the extent of liver regeneration in rats is increased during pregnancy. The study was also extended to screening the action of thalidomide on the oxygen uptake, glucose utilization, and glycogen content of the isolated rat diaphragm.

Male Holtzman rats were partially hepatectomized by the procedure of Higgins and Anderson,[5] leading to the removal of about two thirds of the organ; the liver was dried to constant weight in an oven at 100°. The animals were maintained in individual cages and Rockland rat meal (controls) or the meal supplemented with 0·030 per cent or 0·20 per cent thalidomide on a weight basis together with water administered *ad libitum* over a period of 10·5 days. The rats were then sacrificed (ether) and the entire livers removed and dried to constant weight. The amount of tissue regenerated or the liver increment was calculated from the dry weights by subtracting the product of the weight of tissue excised at surgery and the factor 0·46 from the weight of the liver at necropsy.[6] In the muscle metabolic studies, paired hemidiaphragms were incubated in Warburg vessels with Stadie and Zapp phosphate–saline media containing 120 mg glucose/100ml, according to the procedure described by the writer.[7] Male rats weighing 140–150 g were starved for 24 hr toward depletion of glycogen. They were sacrificed by swift decapitation and the hemidiaphragms removed and processed.

Body weights, liver increments, and the corresponding Fisher $t$ values appear in Table 1. Body

TABLE 1. EFFECT OF THALIDOMIDE ON LIVER REGENERATION IN PARTIALLY HEPATECTOMIZED MALE RATS

Duration: 10·5 days.

| Diet | No. of rats | Body weight (g ± S.E.) | | Liver increment (g ± S.E.) | $t$ |
|---|---|---|---|---|---|
| | | Initial | At necropsy | | |
| Control | 15 | 272 ± 5·7 | 295 ± 6·7 | 2·039 ± 0·088 | |
| Thalidomide (0·030%) | 12 | 275 ± 6·0 | 302 ± 6·0 | 2·276 ± 0·099 | 1·80 |
| Thalidomide (0·20%) | 15 | 264 ± 6·9 | 286 ± 5·5 | 2·473 ± 0·103 | 3·19* |

* $P < 0·01$.

weights were well maintained in each of the groups, and microscopic examination of hematoxylin and eosin-stained liver sections showed rather mild or minimal changes except for one rat in the 0·030%-supplemented series but more widespread alterations with the animals fed the 0·20% ration. Of the latter, half displayed variation in cellular and nuclear size of differing intensity, hyperchromatism, and a few prominent nucleoli; in one section, a focal area of necrosis surrounded by mixed acute inflammatory cells was also present. The increase in the extent of liver regeneration was not statistically significant with the 0·030% ration but quite pronounced with the 0·20% diet. A correlation might be construed between the fetal abnormalities or toxicity and liver regeneration.

An unequivocal mechanism for the increased liver hyperplasia engendered by thalidomide cannot be advanced at present, let alone for the usual mode of tissue restoration in partially hepatectomized animals. If one considers the diversity of agents that can stimulate such growth, including, among others, ingestion of whole liver, coramine, or thiouracil, and injection of carcinogenic hydrocarbons, as well as the stimulation of such growth in pregnancy, the action of this agent may be a direct one on the organ. Another possibility under study by this laboratory is a humoral approach, namely, that the drug may block blood-borne growth decelerators which are generally more prominent under control or quiescent conditions and thereby lead to an imbalance of circulating growth accelerators and, accordingly, to an accentuated anabolism. It might be contended that, since teratogenic effects have been shown to occur with thalidomide in the mouse or rabbit but not in the rat, the findings of King and Kendrick[3] notwithstanding, the rat might have constituted a poor species of choice for these liver studies. However, the alterations produced by the agent might have been so severe or embryocidal as to contribute to the observed resorption, a point brought out for the rat by Christie[8] and for the monkey by Lucey[9]. The above liver findings may substantiate this contention.

Thalidomide employed as a suspension containing microgram amounts up to a level of 0·25 mg in saline was without effect on oxygen and glucose uptakes and the glycogen content of the isolated rat diaphragm (Table 2). The negative respiratory findings are in agreement with earlier accounts. Thus, oxygen consumption by *Tribolium confusum*[15] or Ehrlich ascites tumor cells[16] was not altered by

TABLE 2. AVERAGE DIFFERENCES IN HEMIDIAPHRAGM OXYGEN AND GLUCOSE UPTAKES AND GLYCOGEN CONTENT IN THE PRESENCE OF THALIDOMIDE (0·25 MG)*

| Determination | Mean difference | $t$ |
|---|---|---|
| $Q_{O_2}$ (μl/mg wet tissue/hr) | 0·03 ± 0·059 | 0·46 |
| Glucose (μg/mg wet tissue/hr) | −0·57 ± 0·350 | 1·63 |
| Glycogen (μg/mg wet tissue/hr) | 0·06 ± 0·082 | 0·78 |

Hemidiaphragms were introduced into Warburg flasks containing 1 ml double-strength Stadie and Zapp medium[10] (final concentration: 0·04 M $Na_2HPO_4$, 0·005 M $MgCl_2 \cdot 5H_2O$ and 0·08 M NaCl, pH 6·9; 120 mg glucose/100 ml) and 1 ml saline (glucose standard) or 1 ml thalidomide–saline suspension. The system was gassed with oxygen and incubated for 1 hr at 37°. Glycogen was precipitated after digestion of the tissue with 30% KOH and determined by Dreywood's reagent.[11, 12] Glucose in the incubation mixture was ascertained after deproteinization.[13, 14]

* The means (± S.E.) are based on 17 paired hemidiaphragms. A positive mean difference indicates a decrease in the presence of the agent; the extent of glucose utilization was based on the final concentration of the respective media incubated without diaphragm.

this compound. However, Frank and co-workers[17] noted that the inhibition of protozoa by thalidomide could be alleviated by nicotinate and vitamin K, suggesting a mechanism possibly bound up with oxidation.

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