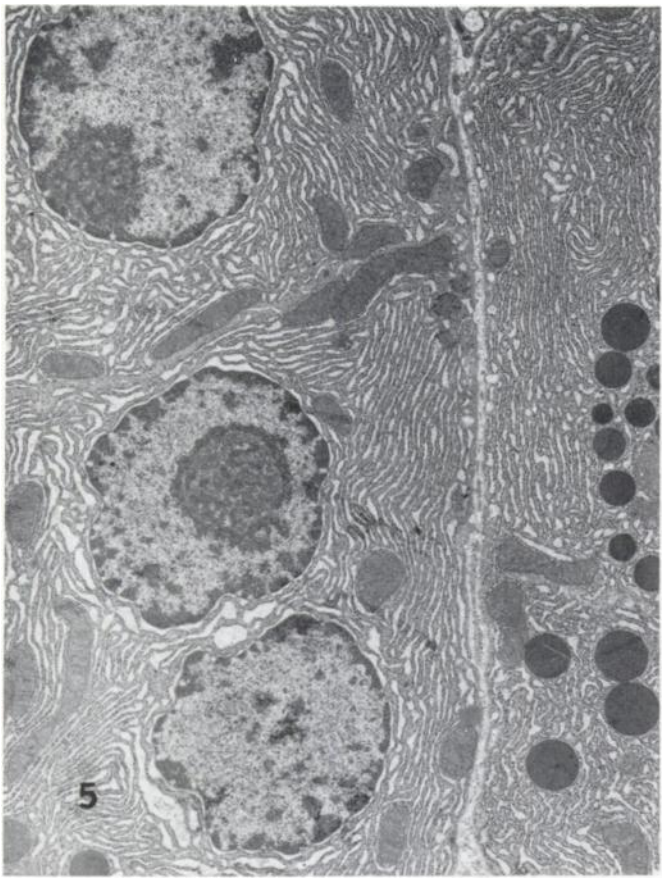

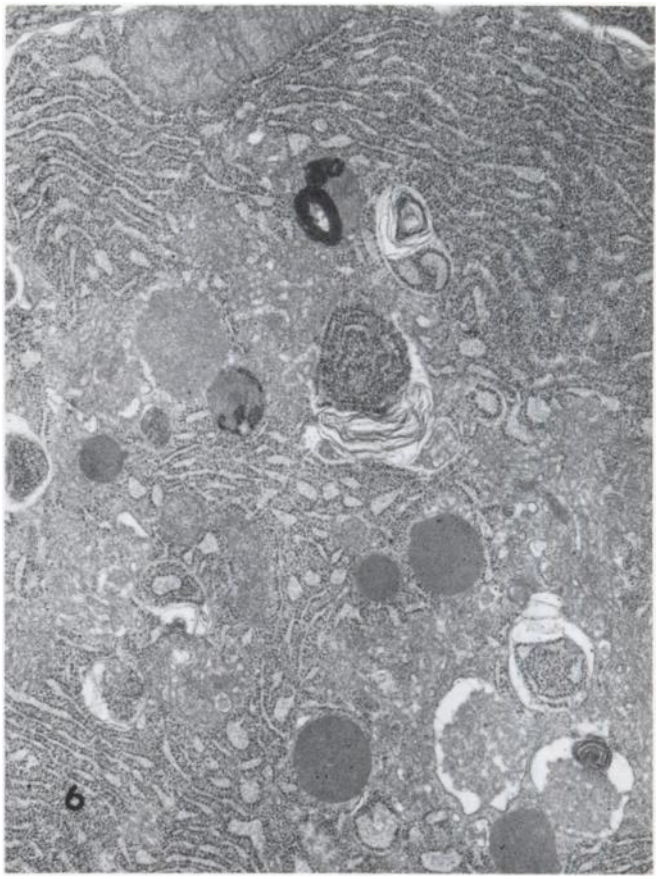

Informal Discussion following the Paper by Scarpelli

Dr. Weisburger: Dr. Scarpelli, we, of course, know you for your participation on the important elucidation of the factors that bore on the induction of hepatoma 10 or 15 years ago. It is interesting that you now come back to 1 of the elements that seem to be involved at this time, and this is really very encouraging. Did you imply that the decreasing effect as you fed sterculic acid in these studies was related to the increasing age? If so, did you use older animals at the higher doses and find it was possible to compensate for this effect?

Dr. Scarpelli: Yes, there is a decreasing mitogenic effect of cyclopropenoid fatty acids with increasing age of the animals both with liver and pancreas. Animals weighing 500 or 600 g will develop a fatty liver but no mitogenic response.

Dr. Weisburger: One is always intrigued with chemicals which have double bonds in them. Do you by chance have access to the corresponding epoxide, because it could be that the effect of age may relate to the fact that the older animals not only have a decreased rate of metabolism generally, but it could also be that they have lower formation of an active metabolite such as the epoxide or, reversely, increased detoxification, through epoxide hydrase or glutathione transferase to detoxified products?

Dr. Scarpelli: That's a very good point. I do not have access to such information. Dr. Sinnhuber of Oregon State University is studying the metabolism of cyclopropenoid fatty acids.

Dr. Longnecker: Do the pancreases achieve a larger size?

Dr. Scarpelli: Yes, they do, It's of course limited, stopping after about 2 months of feeding.

Dr. Longnecker: We have recently observed the effects of treating animals between the weights of 50 and 250 g with the effects of starting with animals that weigh 250 g. We have many fewer nodules in the older rats.

Dr. Alarif: I was just wondering about the type of compound you used. Was it the methyl ester or the free acid?

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Dr. Scarpelli: I have used the triglycerides of sterculic and malvalic acids as they are present in *Sterculia foetida* oil. This is incorporated in the diet at a level of 500 ppm. We have demonstrated that the methyl ester of sterculic acid is, indeed, one of the mitogens since mitosis can be induced in liver cells when it is administered as a single dose.

Dr. Farber: Dr. Scarpelli, it is very critical to know whether this is a primary stimulatory event or is merely secondary to cell injury. As you have pointed out, there are selected areas of necrosis. Do you think they could account possibly for the initiation of a regenerative response?

Dr. Scarpelli: Initially, in liver and again in pancreas, there is mitosis with no morphological evidence of cell injury. As one continues to feed these compounds, focal liver cell and pancreatic acinar cell injury develops. In the liver, about 0.45% of the liver cells were necrotic after 4 weeks of feeding at a time when the mitotic index was considerably higher. Earlier, there is mitosis without necrosis, with a single dose of the methyl ester administered by stomach tube; there are numerous mitoses in the absence of necrosis.

Dr. Farber: Did you test for necrosis only histologically, or did you measure it chemically as well, such as serum enzyme levels or release of some label, *e.g.*, iodine as iododeoxyuridine from the DNA in damaged cells?

Dr. Scarpelli: We have not tested for early evidence of cell injury by chemical means, but we do intend to do this.

Dr. Farber: The chemical index is probably more sensitive than the histological index.

Dr. Pledger: Dr. Scarpelli, is there any estimate of the amount of contact the human may have with cyclopropenoid fatty acids through direct consumption or via a food-providing animal vector?

Dr. Scarpelli: This material is used as a food source by large segments of the world population. These compounds are present in cottonseed oil and meal in amounts which are capable of inducing mitosis in rat liver and pancreas. However, the implications for man are impossible to assess since there are no data concerning their effects on primates. One additional point of interest is that the body fat of animals fed diets containing cottonseed meal that has not been rid of cyclopropenoid fatty acids contains these acids; apparently, these are incorporated into tissue lipids. Preliminary studies suggest that cyclopropenoid fatty acids are incorporated into membrane lipids. Thus they may prove useful as a probe to study how alterations of cell membranes induce mitosis in cells, like adult liver, most of which are normally nondividing or in the G_0 phase so that there is a very low mitotic index on the order of 0.1 to 0.2 per 1000 hepatocytes.

Dr. Weisburger: Animal feed may contain these materials as residues during the production of cottonseed oil. However, at the time of the trout hepatoma studies, we looked into the problem of oil produced for human consumption. We found that the cottonseed oil, as used in the human situation, does not contain any of these materials. Would that be true, Dr. Scarpelli?

Dr. Scarpelli: It varies from batch to batch. We have found cyclopropenoid fatty acids in certain batches of a commercially available cottonseed oil at levels as high as 0.3%; other batches may be entirely free of them. There are, incidentally, some strains of cotton that have much less of this material in its seed than others, and perhaps this coupled with rigorous extraction by hexane and steam may be the answer to removing effectively such substances from the diet of humans.

Dr. Farber: Do you know whether this works in tissue culture?

Dr. Scarpelli: I have not tried it in tissue culture. I have sent Dr. Henry Pitot a sample, and he will test it on some liver cell cultures which, as you know, do not divide spontaneously.

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