[*Journal of Research in Indian Medicine* (1974): (9), 2, 60-62]

Effect of Liv.52 on Regeneration of Liver Cells in Tissue Culture (A Preliminary Report)

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Tissues in the body constantly require nutrients and oxygen, which are supplied by the blood. They also remain in optimum working condition at normal body temperature. Tissues removed from the body lose their function and perish rapidly. But by the development of tissue culture, science has made it possible to create conditions in the test tube, which can maintain the life of the tissue cells. Not only this but under appropriate conditions the tissue cells can maintain their function and capability of growth and multiplication. The advantage of using tissue cultures to study drug effects is that a very small amount of the drug is necessary and the effects can be studied directly on the tissue cells at different intervals and both structural and functional effects can be elucidated. The technique of tissue culture is very rigorous and utmost precautions have to be taken in the matter of sterility and accuracy in preparing culture media. Also, all factors like temperature and osmolarity have to be maintained similar to normal tissue bathing fluid. But with reasonable care, it is now possible to use this technique for study of drugs on various adult and embryonic tissues like the liver.

Normally there is no difficulty in maintaining tissues obtained from normal livers but tissues obtained from livers but tissues obtained from cirrhotic livers show predominance of fibroblasts with very little growth of parenchyma. There is also accumulation of yellow pigment indicating functional impairment (Gay and Bang, 1951).

Liv.52 has been shown in various animal models as well as clinical trials to cause the regeneration of healthy liver parenchyma. It was therefore decided to study the effect of Liv.52 on embryonic and abnormal adult liver tissue, using tissue culture techniques.

MATERIAL AND METHODS

In this study, experiments were carried out both on chick embryonic liver and also on diseased (liver cirrhosis) livers of patients. Thus the study was divided into two groups i.e. (1) Effect of Liv.52 on embryonic liver, (2) on adult (human) diseased liver.

CULTURE TECHNIQUE

Group I: Fourteen days old chick embryonic liver was dissected out and cut into immersed in tyrodes solution throughout the dissection. After washing the pieces several times with tyrodes solution, approximately two equal pieces were selected. They were kept on the stainless steel grind tablet lying in a small culture vessel. One piece was kept as control and the other piece was treated with Liv.52. These small culture vessels were kept in a bigger petri dish containing normal saline and then impregnated in a McIntosh jar and incubated at 38.5^{o}C.

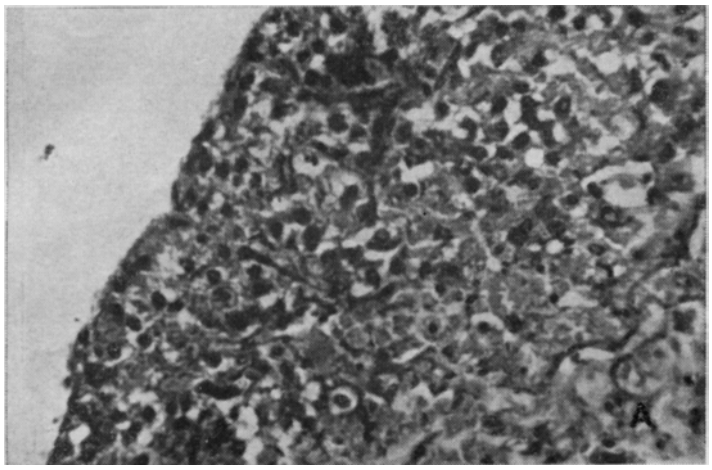
Group II: In this, two cases of proved liver cirrhosis were selected. The specimens were collected by needle biopsy under totally sterile conditions. They were transferred from the hospital to the laboratory under tyrode solution and cultivated immediately *in vitro* as described above.

Culture Media

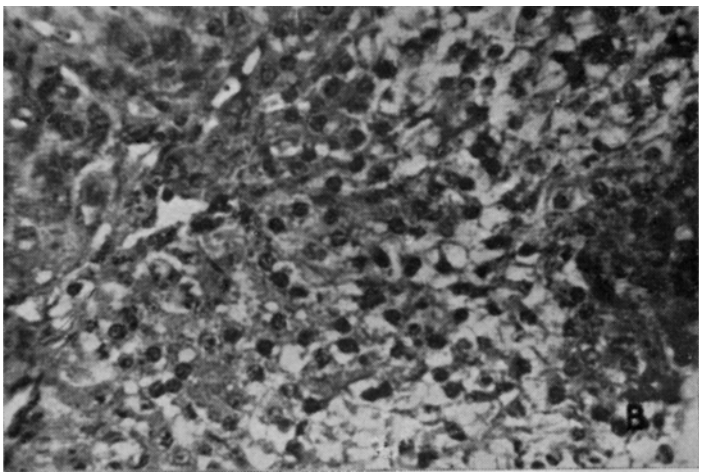
The culture media used in this study was protein-free, chemically defined medium as described by Prasad and Reynolds (1968) with slight modification. Liv.52 powder (received from The Himalaya

Drug Co. Pvt. Ltd., Bombay) was dissolved in double distilled water and the water soluble fraction was added into the culture medium in the dose of 0.84 g/ml of the medium. The medium was changed every alternate day.

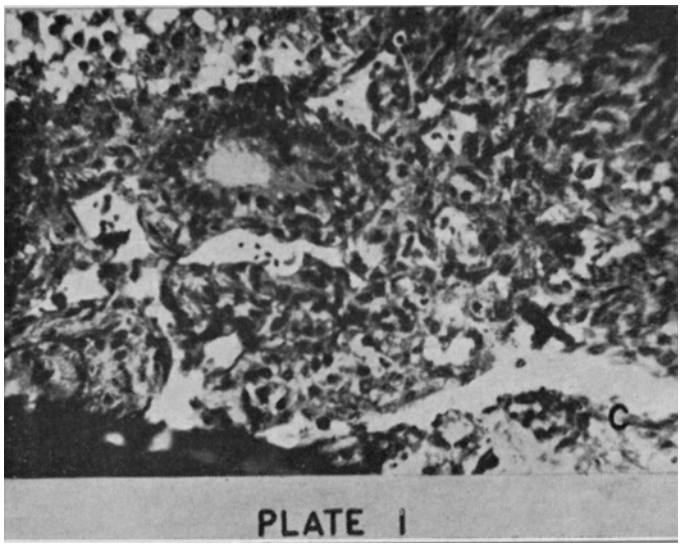
Control



P.A.S. stain of explant of chick liver grown by tissue culture shows positive for accumulation of glycogen granulus. Some cells are vacuolated and the biliary and hepatic ducts are not well visualised.

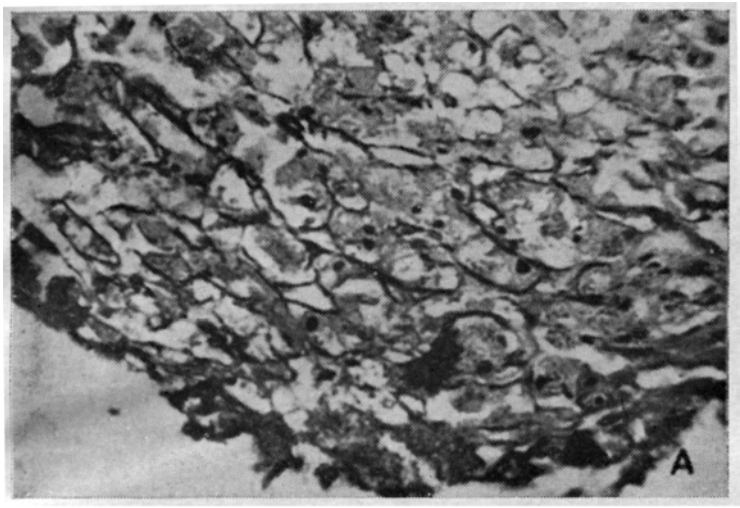


With Liv.52 added to the medium.

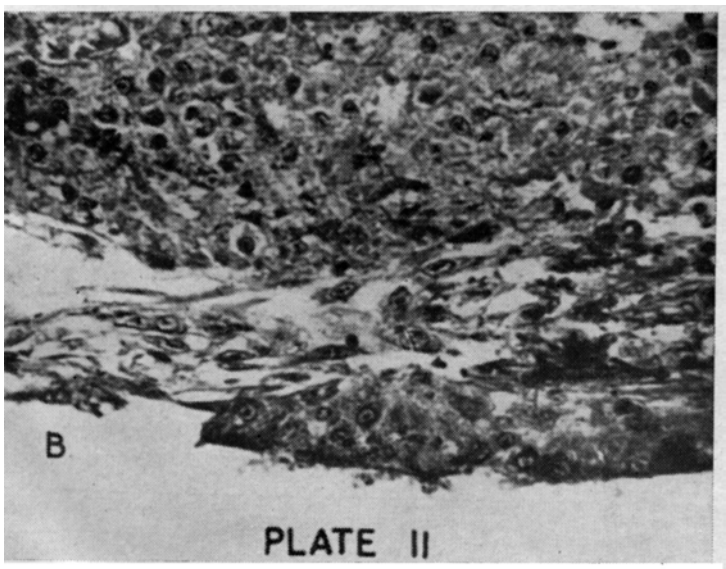


Explant of chick liver grown by tissue culture shows, in slides B and C, a fair amount of haemopoietic factor with well maintained hepatic ducts and liver parenchyma cells.

CONTROL



Cirrhotic human liver. Explant grown by tissue culture seen in the early period shows many fibrotic bands in the parenchymatous cells.



Cirrhotic human liver. Explant grown by tissue culture seen later shows that fibrotic bands have dissolved and there are some signs of growth.

RESULTS

Effect on Chick Liver: Results of the experiments carried out on chick embryonic liver cells showed that the explants were healthy up to the end of 4 days *in vitro*. As the culture became older, the tissue became somewhat paler in colour whereas the explant cultivated in the medium containing Liv.52 showed that the tissue was healthy throughout the culture period without any change in the colour.

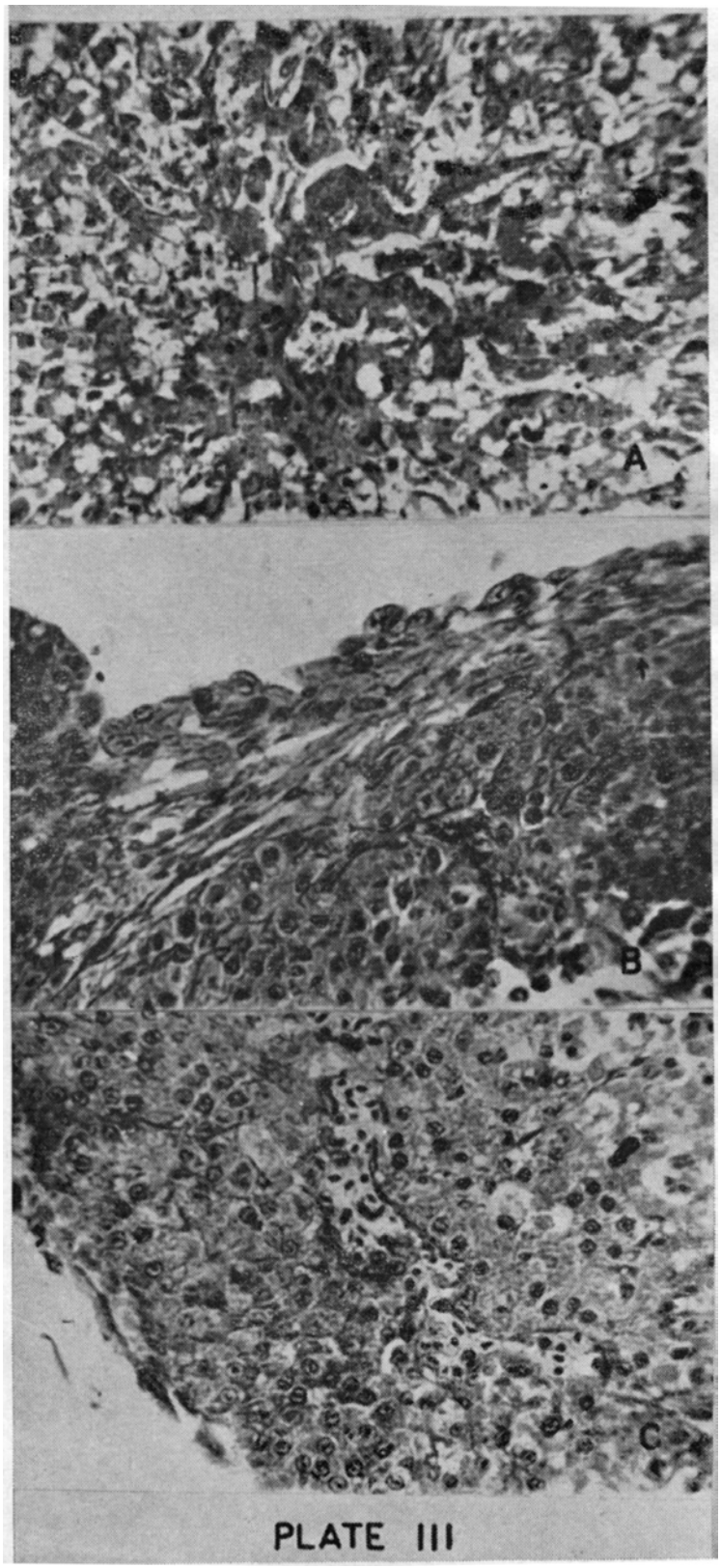
Histological studies of the control revealed that the haemopoietic factor was maintained with a fair amount of liver parenchyma cells. Some of the cells became vacuolated. The biliary and hepatic ducts were not well visualised. P.A.S. staining showed positive for accumulation of glycogen granulus (Plate I, Fig. A). Whereas the histological picture of similar explants grown in the medium containing Liv.52 showed no very significant change except that there was a fair amount of haemopoietic factor, with well maintained hepatic duct and liver parenchyma cells (Plate I, Figs. B and C).

The biochemical studies revealed that there was more of cholesterol and bilirubin in the medium on the second day i.e. 36.5 mg% and 0.44 mg% respectively on the 2nd day after culture followed by decline in the level on the fourth day of culture i.e. cholesterol came down to 28.3 mg% and bilirubin to 0.28 mg%. Whereas the explant grown in the medium containing Liv.52 showed a greater decrease in the cholesterol and bilirubin content on the 2nd day i.e. 24.5 mg% and 0.25 mg% respectively and on the 4th day 24.3 mg% and 0.23 mg% respectively.

This suggests that probably *in vitro* there was more production of bilirubin in the early period by the liver cells, which becomes less during the later period. The decrease in the later period might be due to the fact that, some of the liver cells may not be functioning as well as they did in the earlier period. The addition of Liv.52 to the medium probably inhibits the deposition or formation of more bilirubin during the early period and maintains the level, as those cells are active and alive up to a later period.

Effect on the Human Liver: The growth of the diseased (cirrhosis) liver in organic culture showed that in the early period of culture, there were many fibrotic bands in the area of parenchymatous

With Liv.52 added to the culture medium



A: Cirrhotic human liver shows dissolution of fibrotic bands

B: Cirrhotic human liver. With Liv.52 added to the culture media. Explant grown by tissue culture shows regeneration of parenchymatous cells with well-maintained haemopoietic factors. In addtion some dividing cells can also be seen-marked by arrows.

C: Arrow shows dividing cells.

cells (Plate IIA). These seem to be dissolved in the later stage of culture (Plate IIIA). There was some sign of growth of the liver cells, but most of the cells could not grow up to the later period; whereas the explant from the same piece of biopsy, grown in the medium containing Liv.52, showed regeneration of parenchyma cells with well-maintained haemopoietic factors up to the end of 4 days of culture. In addition to this, some dividing cells also could be seen (marked with an arrow, Plate IIB and Plate III B and C).

This suggests that probably Liv.52 stimulates the mitotic activity of the cells and so stimulates the liver cells to regenerate. However, to confirm the preliminary findings, a detailed study should be carried out.

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