Br. J. exp. Path. (1981) **62**, 227

JAUNDICE AND WOUND HEALING: A TISSUE-CULTURE STUDY

M. TAUBE, P. ELLIOT AND H. ELLIS

From the Surgical Unit, Westminster Hospital, Dean Ryle Street, London S.W.1

Received for publication January 15, 1981

Summary.—The effects of jaundice on wound healing have been studied by growing fibroblasts, *in vitro*, in normal culture media, in culture media to which bilirubin has been added, and in culture media containing sera from jaundiced patients. It has been found that the addition of bilirubin to the culture media causes morphological changes in the fibroblasts, and impairs the growth of cells. The addition of jaundiced human sera to the culture media also causes similar changes.

THERE IS MUCH anecdotal evidence that jaundice impairs wound healing. However, actual experimental data remains limited. Ellis and Heddle (1977) showed that there was an increased risk of wound dehiscence and incisional hernia in jaundiced patients, and Bayer and Ellis (1976) demonstrated that healing in abdominal wounds and angiogenesis in gastric incisions were delayed in rats with obstructive jaundice.,

However, it has recently been suggested by Irvin *et al.* (1978) that any increased tendency towards wound failure is only seen in those patients with jaundice caused by malignancy. They postulate that other biochemical abnormalities such as hypoproteinaemia, associated with jaundice, may be the cause of these wound failures.

Our study was specifically designed to test the hypothesis that jaundice, *per se*, impairs wound healing. In order to do this we have grown rat fibroblasts, *in vitro*, in normal culture media and in culture media to which bilirubin has been added. We have also grown rat fibroblasts in culture media containing human sera obtained from normal subjects and in sera obtained from patients who were jaundiced from a variety of causes.

Reprint requests to H. Ellis.

METHODS

The method used was similar to that described by Colin, Elliot and Ellis (1979) from this laboratory. The abdominal wall of Wistar rats was prepared by shaving off the hairs and applying Nystatin cream. Small pieces of skin were removed and further trimmed of fat and hair under sterile conditions. The skin was cut into 1 mm squares and placed in tissue-culture dishes containing TC 199 (Hepes buffer) with 10% added foetal calf serum (FCS). The small pieces of skin were kept flat on the bottom of the tissue-culture dishes by means of glass cover slips. The skin was incubated for about 5 days until outgrowth of fibroblasts into the surrounding area was obvious. These fibroblasts were removed and seeded in Falcon bottles containing the same culture medium. They were allowed to continue growing for several weeks in these bottles. A known number of cells, estimated by means of a counting grid, were removed and placed in multiple tissue-culture wells. The tissue-culture wells and their contents were incubated at 37° for 2 days, until the fibroblasts had become adherent to the plastic surface of the tissue culture wells. The culture medium was then removed and replaced by culture media consisting of TC 199 and 10% FCS with bilirubin added at the following concentrations: 0, 10, 20, 42, 85, 140 and 170 μmol/l. In some wells the normal culture medium was replaced by new media in which the 10% FCS was replaced by human sera. Some of the human sera were from normal healthy subjects, whilst some were from patients who were jaundiced in varying degrees (ranging from 65 to 412 μmol/l of bilirubin). The jaundice in these patients was

TABLE I.—*Details of patients from whom sera were obtained*

Age	Sex	Cause of jaundice	Bilirubin concentration (μmol/l) Total	Conjugated	Mean no. of cells at one week N = 20	Standard deviation
21	M	Control	< 17		4968	164
21	M	Control	< 17		4809	390
20	F	Control	< 17		4489	512
73	M	Carcinoma of head of the pancreas	412	265	1892	361
64	F	Gallstones in the common bile duct	112	65	4116	461
23	M	Hodgkins disease. ? nodes at the porta hepatis	67	36	2097	426
47	M	Cirrhosis of the liver	106	51	2948	459
66	F	Carcinoma of the breast. Hepatic secondaries	212	164	1564	586
68	F	Carcinoma. Unknown primary. Hepatic secondaries	65	45	3828	436
73	M	Carcinoma of head of the pancreas	248	171	2962	513

caused by a variety of diseases. All the patients had normal concentrations of urea and proteins in their sera. They were not receiving cytotoxic therapy (Table I). The cells were then incubated for a further week at 37°, during which time any morphological changes which occurred were recorded. After this the cells were freed from the walls of the plastic dishes by the addition of a small quantity of trypsin solution. The number of cells present in each well was counted in a Coulter counter.

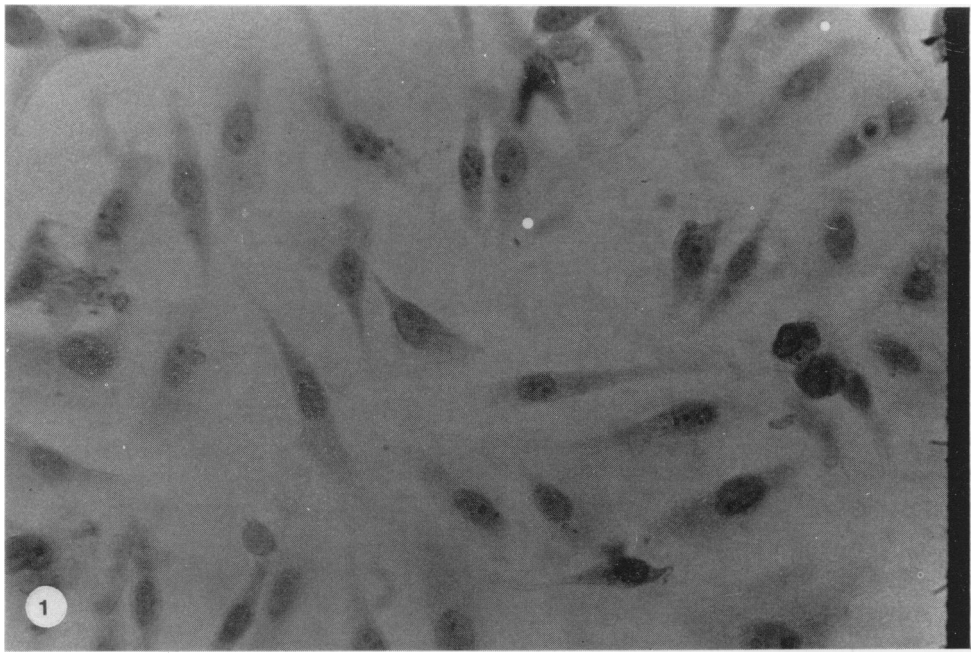
RESULTS

A marked morphological change was observed in the cells that were grown in culture media which contained bilirubin. These changes were easily seen with a light microscope, and became obvious when the concentration of bilirubin exceeded 85 μmol/l. Normal fibroblasts are fusiform in shape, and when grown in plastic dishes align themselves in abundant sheets of parallel cells. The fibroblasts in the culture media containing high concentrations of bilirubin were sparse, irregular in shape and were typically much more rounded. There was also a marked loss of cytoplasm in these cells and there was loss of the normal parallel alignment of the cells. There was a tendency for the cells to lose their adhesion to the sides of the plastic dishes (see Figs 1 and 2).

TABLE II.—*Number of cells present in each group of tissue culture wells after 1 week of incubation. N = 14*

Concentration of bilirubin μmol/l	Mean number of cells at start	Mean number of cells at 1 week	Standard deviation
0	2000	4306	491
10	2000	4174	533
20	2000	4002	591
42	2000	3023	651
85	2000	2804	554
140	2000	1229	170
170	2000	1822	307

Experiments in which the cells were grown in culture media containing bilirubin and counted at the end of 1 week's incubation showed that even in low concentrations of bilirubin (42 μmol/l) there was an obvious inhibition of growth. At higher concentrations of bilirubin in the culture media (in excess of 85 μmol/l) the number of cells present in each well at the end of 1 week was in fact less than the number of cells present at the start. This implies a cytotoxic effect. An approximately linear relationship was found to exist between the number of cells present



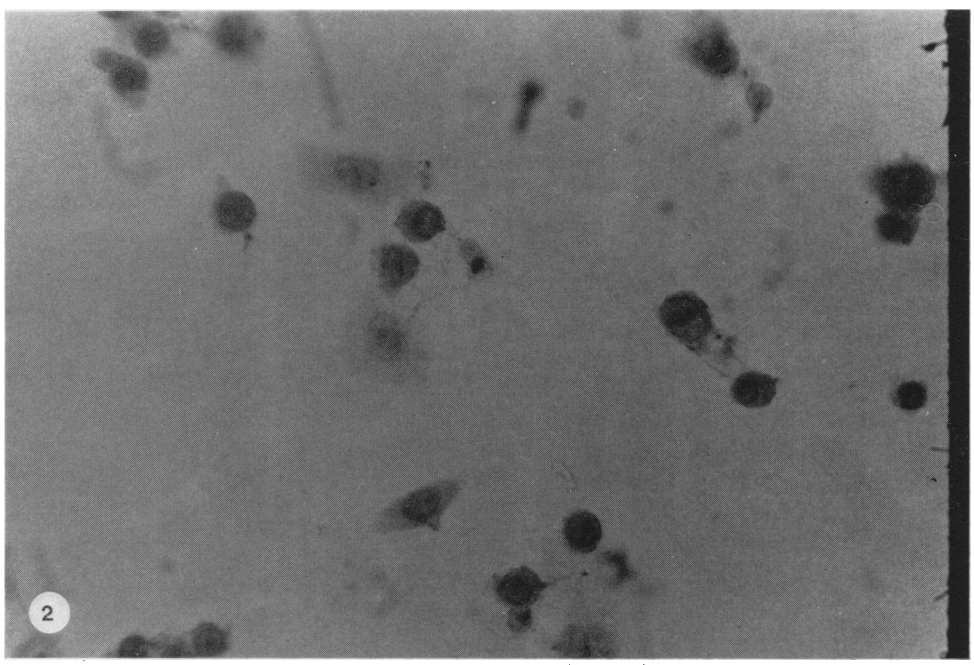


FIG. 1.—Normal fibroblasts growing *in vitro*. The cells are fusiform and aligned in rows. ×400.
FIG. 2.—Fibroblasts in culture medium containing bilirubin at a concentration of 140 μmol/l. The cells are sparse irregular and show loss of cytoplasm. ×400.

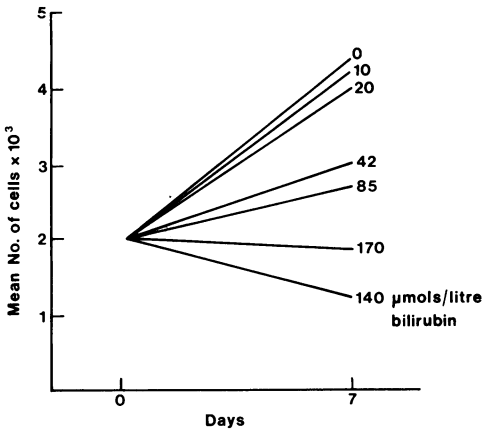


FIG. 3.—The differential rate of growth of fibroblasts at varying concentrations of bilirubin (µmol/l). N = 14.

after incubation for 1 week and the concentration of bilirubin in the culture media (see Figs 3 and 4).

In our experiments in which the FCS was replaced by human sera, there was no obvious difference between those cells grown in medium containing 10% FCS and those grown in medium containing 10% normal human serum. However, growth was less in those cases in which the serum was from jaundiced patients. Morphological changes were also noted. The degree of growth inhibition that occurred when the cells were grown in media containing jaundiced sera did not correlate well with the concentrations of bilirubin in the jaundiced sera. However, the number of patients involved was

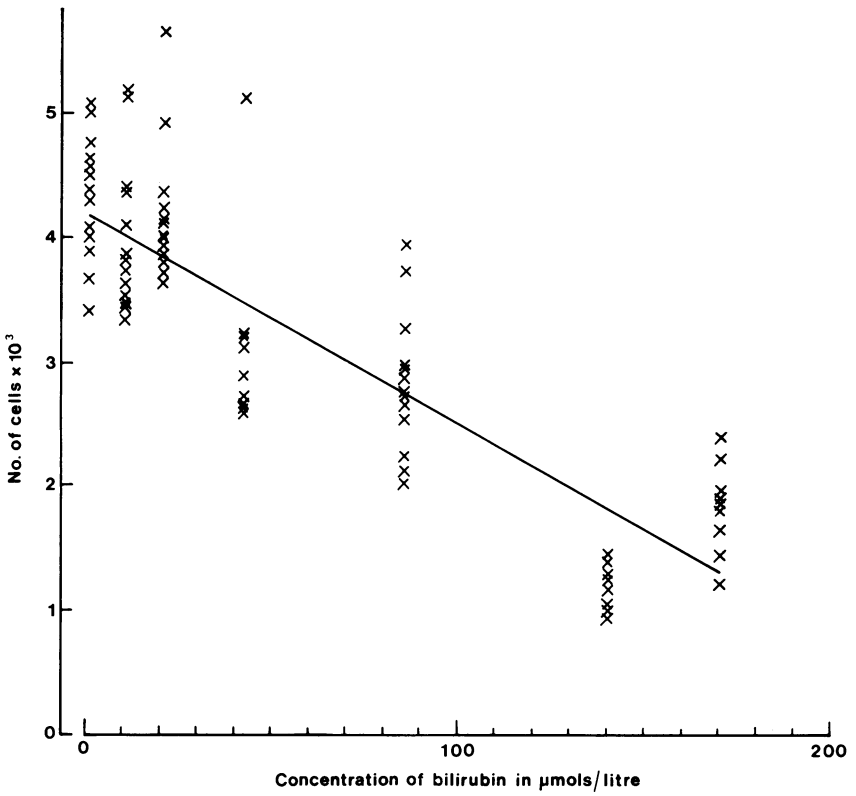


FIG. 4.—Scatter diagram showing number of cells present after 1 week of incubation in culture media containing different concentrations of bilirubin. N = 14 for each concentration of bilirubin. Spearman Rank Correlation Coefficient = −0·852 ($P > 0·0001$). Regression line intercept (Y on X) = 4181. Gradients = −16·93.

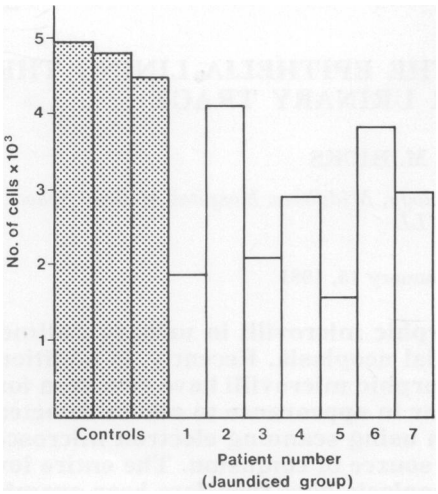


FIG. 5.—Histogram showing mean number of cells present after 1 week of incubation in culture media containing 10% serum from normal and jaundiced patients (±1 s.d. N=20).

small, and it is not possible to state that one cause of jaundice has a more marked growth inhibition effect than another (Fig. 5).

DISCUSSION

Our results have shown that bilirubin inhibits the growth of fibroblasts, *in vitro*, even in concentrations that are only slightly above normal. Fibroblasts die in culture media containing concentrations of bilirubin above 170 μmol/l. Fibroblasts do not grow well in sera from patients with jaundice from various causes. In view of the central role of fibroblasts in wound healing there can now be little doubt that jaundice, *per se*, has an adverse effect on wound healing.

Our results correlate well with the work of several other authors. Lee (1972) showed that the migration of reticulo-endothelial cells and fibroblasts into experimental granulomata was inhibited in jaundiced rats. Than Than (1976) showed that prolyl hydroxylase activity was decreased in the skin of jaundiced rats. Karakantzas (1975) has showed that there is a delay in healing of wounds of the parietal peritoneum in rats with obstructive jaundice. This was confirmed by Bayer and Ellis (1976). More recently Greaney *et al.* (1979) have shown that there is a delay in the accumulation of collagen in abdominal wounds of jaundiced rats.

Our observations on the morphological changes that occurred suggest that the site of the toxic action of bilirubin may well be on the membrane of the fibroblast, as the cells were seen to swell, become rounded, and lose their adherence to the walls of the culture dishes. However, more work is required in this respect, and we hope to identify more clearly the site of this effect in future studies. It is not possible at this stage to be certain whether the cause of the jaundice, as well as the degree of jaundice, is quantitatively important in the impairment of wound healing.

REFERENCES

BAYER, I. & ELLIS, H. (1976) Jaundice and Wound Healing: An Experimental Study. *Br. J. Surg.*, **63,** 392.

COLIN, J. F., ELLIOT, P. & ELLIS, H. (1979) The Effects of Uraemia upon Wound Healing: An Experimental Study. *Br. J. Surg.*, **66,** 793.

ELLIS, H. & HEDDLE, R. (1977) Does the Peritoneum Need to be Closed at Laparotomy? *Br. J. Surg.*, **64,** 733.

GREANEY, M. G., VAN NOORT, R., SMYTHE, A. & IRVIN, T. T. (1979) Does Obstructive Jaundice Adversely Affect Wound Healing? *Br. J. Surg.*, 478.

IRVIN, T. T., VASSILAKIS, J. S. & CHATTOPADHYAY, D. K. (1978) Abdominal Wound Healing in Jaundiced Patients. *Br. J. Surg.*, **65,** 521.

KARAKANTZAS, D. (1975) The Effects of Obstructive Jaundice on Wound Healing: An Experimental Study. Thesis. University of Thessalonika, Greece.

LEE, E. (1972) The Effects of Obstructive Jaundice on the Migration of Reticulo-endothelial Cells and Fibroblasts into Early Experimental Granulomata. *Br. J. Surg.*, **59,** 875.

THAN THAN (1976) Skin Prolyl Hydroxylase and Tensiometry in Janudice. Ph.D. Thesis, University of Glasgow.