Prolyl hydroxylase in the skin of patients with obstructive jaundice

THAN THAN, J. O'D. MCGEE¹, AND L. H. BLUMGART

From the University Departments of Surgery and Pathology, Glasgow Royal Infirmary and the Department of Pathology¹, Radcliffe Infirmary, Oxford, UK

SUMMARY Prolyl hydroxylase was estimated in the skin of 24 jaundiced patients and 25 non-jaundiced patients. The activity of this enzyme was markedly reduced in the skin of the jaundiced patients.

It has long been the impression of surgeons that wound healing is impaired in patients with obstructive jaundice, particularly when this is associated with malignant disease (Torrance, 1972). However, there is no conclusive evidence to support this belief although it has been shown experimentally that migration of fibroblasts and granuloma formation are abnormal in rats with obstructive jaundice (Lee, 1972). More recently, Ellis and his colleagues (Bayer and Ellis, 1976) have suggested that wound healing is defective in jaundiced rats.

In a preliminary communication (Than Than *et al.*, 1974), we reported that prolyl hydroxylase, which catalyses the synthesis of hydroxyproline in collagen, was markedly reduced in the skin of a small group of patients with obstructive jaundice. This paper reports a more extended study of skin prolyl hydroxylase activity in three groups of patients with obstructive jaundice.

Material and methods

MEASUREMENT OF SKIN PROLYL HYDROXYLASE

Skin prolyl hydroxylase was measured as previously reported (Than Than *et al.*, 1974). After informed consent had been obtained a specimen of skin was taken either from the edge of an upper midline or right upper paramedian incision at the time of operation or, in patients not submitted to laparotomy, at the site of a needle biopsy of the liver. Fat was carefully removed from the skin specimen which was then wrapped in aluminium foil and stored for assay at -20°C . Assays for prolyl hydroxylase were carried out in three batches of patients, and for each batch substrate was prepared by a modification of the

method of Hutton *et al.* (1966). The efficiency of the substrate prepared differed from batch to batch, and the results of the assays are therefore not comparable between batches. At the time of assay approximately 0.5 g of skin was homogenised, as previously described (Than Than *et al.*, 1974), the homogenates being centrifuged at 20 000 *g* for 15 minutes for the first two batches of patients and at 15 000 *g* for 15 minutes for the third batch. Prolyl hydroxylase activity was measured in duplicate by the tritium release technique (Hutton *et al.*, 1966) in a modified reaction mixture (McGee *et al.*, 1974). Proteins in the skin supernatants were estimated by the method of Lowry *et al.* (1951). Prolyl hydroxylase levels are expressed as counts per minute per milligram of protein.

PATIENTS

Patients in all three batches consisted of those with jaundice consequent on benign or malignant obstruction of the common bile duct or as a result of alcoholic or primary biliary cirrhosis. Control studies were carried out in non-jaundiced patients submitted to laparotomy for surgical treatment of peptic ulcer or gall bladder disease, and in one patient for aneurysm of the abdominal aorta.

Plasma proteins, serum bilirubin, serum aspartate aminotransferase (SGOT), serum alanine aminotransferase (SGPT), and alkaline phosphatase were measured by routine laboratory methods.

STATISTICAL ANALYSIS

Since a fairly wide scatter of results with a correspondingly large standard deviation has been found in our studies of skin prolyl hydroxylase and since the results do not display a normal distribution the Mann-Whitney non-parametric test with Wilcoxon's U-statistics (Campbell, 1974) was used to analyse the

Received for publication 31 March 1977

results. This test compares possible low values (in this case low levels of serum prolyl hydroxylase in jaundiced patients) with all possible combinations of higher values in control subjects.

Results

The median value for skin prolyl hydroxylase activity in the jaundiced patients was markedly lower than that of the controls in all three batches of patients (Tables 1, 2, and 3). Indeed, in batches 2 and 3 prolyl hydroxylase activity was approximately 50% of that

of the control group whereas in batch 2 (Table 2) it was only one-third of that of the control. Statistical analysis, however, revealed that there was a significant difference between the jaundiced and control groups only in series 2 and 3, series 1 failing to reach statistical significance. There was no detectable difference in prolyl hydroxylase activity between patients jaundiced as the result of bile duct obstruction or as a result of liver disease, and there appeared to be no difference either between patients with benign or malignant obstruction of the common bile duct.

Table 1 Skin prolyl hydroxylase (PH) activities in control and jaundiced patients (series 1)

Group	No.	Age	Diagnosis	Skin PH (cpm/mg protein)	Median
Controls	1	45	Peptic ulcer	113	320
	2	68	" "	113	
	3	59	" "	527	
	4	56	" "	888	
Obstructive jaundice	1	69	Carcinoma head of pancreas	24	151.1
	2	73	Gall stones	103	
	3	55	Cholecystitis	116	
	4	69	Gall stones	187	
	5	78	Gall stones + chronic cholecystitis	333	
	6	74	Carcinoma head of pancreas	464	
Alcoholic cirrhosis	1	50	Acute alcoholic liver disease	116	161
	2	58	Alcoholic cirrhosis	206	
Primary biliary cirrhosis	1	58	PBC	128	166
	2	54		166	
	3	44		225	

Each prolyl hydroxylase result represents the mean of two estimations.

Table 2 Skin prolyl hydroxylase (PH) activities in control and jaundiced patients (series 2)

Group	No.	Age	Diagnosis	Skin PH (cpm/mg protein)	Median
Controls	1	65	Chronic cholecystitis	76	365
	2		" "	104	
	3		Peptic ulcer	153	
	4	23	Gall stones	161	
	5		Peptic ulcer	197	
	6		" "	259	
	7	19	Gall stones	259	
	8	39	Chronic cholecystitis	365	
	9	81	Gall stones	421	
	10	53	Peptic ulcer	538	
	11		Chronic cholecystitis	581	
	12		Gall stones	731	
	13	31	Chronic cholecystitis	936	
	14	35	Peptic ulcer	1169	
	15	42	Chronic pyloric ulcer	1340	
Obstructive jaundice	1	22	Choledochal cyst	16	121
	2	75	Gall stones	25	
	3	31	Carcinoma pancreas	43	
	4		Secondaries in hepatic nodes	64	
	5		Gall stones	111	
	6	49	Acholangitis	131	
	7	37	Carcinoma common bile duct	150	
	8	70	" " " "	493	
	9	82	Gall stones	530	
	10	61	Carcinoma common bile duct	558	

Each prolyl hydroxylase result represents the mean of two estimations.

Table 3 Skin prolyl hydroxylase (PH) activities in control and jaundiced patients (series 3)

Group	No.	Age	Diagnosis	Skin PH (cpm/mg protein)	Median
Controls	1	55	Aortic aneurysm	38	50-5
	2	25	Duodenal ulcer	39	
	3	21	Gall stones	50	
	4	55	Duodenal ulcer	51	
	5		Jejunal ulcer	52	
	6	69	Gall stones	69	
Jaundice	1	65	Gall stones	21	22
	2	36	" "	22	
	3	60	Carcinoma head of pancreas	36	

Each prolyl hydroxylase result represents the mean of two estimations.

The total protein contents in the skin supernatants of both jaundiced and control patients were not different from each other and only four of the total of 24 jaundiced patients had a serum albumin level below 3 g/100 ml. No correlation was found between serum bilirubin level, serum albumin level, SGOT, SGPT or alkaline phosphatase and the level of the skin prolyl hydroxylase.

Discussion

The findings we report are in accord with the results of our preliminary report (Than Than *et al.*, 1974), namely, that there is a reduction in prolyl hydroxylase in the skin of jaundiced patients. Prolyl hydroxylase is responsible for the synthesis of hydroxyproline in collagen and hydroxyproline stabilises the collagen triple helix (Jiminez *et al.*, 1973). A reduction in skin level of this enzyme in jaundice could conceivably lead to a diminution in the stability of skin collagen. On the other hand, it may be that collagen synthesis is generally depressed and that the low levels of prolyl hydroxylase are an index of this. Either defect in collagen synthesis might account for the clinical impression of poor wound healing in jaundiced patients.

It should be remembered, however, that biliary obstruction may be associated with a degree of nutritional deficiency and often with biliary tract infection, and these factors must be taken into account. It is, however, interesting to note that only four of the 24 jaundiced patients studied had a serum albumin level below 3 g/100 ml. Moreover, no correlation could be demonstrated between the serum albumin level and the decrease in the prolyl hydroxylase activity in the jaundiced patient.

It is interesting to note that the serum bilirubin level could not be correlated with the decrease in

prolyl hydroxylase activity. It seems unlikely that bilirubin itself would have an effect in lowering the prolyl hydroxylase activity and indeed we have found that sera containing high levels of added bilirubin had no effect in lowering the prolyl hydroxylase activity *in vivo* (Than Than *et al.*, in preparation). It is possible that a local action of retained bile salts might be responsible but we have no evidence of this at present.

The evidence we present suggests that jaundice is associated with low skin prolyl hydroxylase, and in patients submitted to operation for obstructive jaundice this might impair healing. A controlled clinical study would, however, be necessary to prove this point. In this respect it is interesting that our animal experiments (Than Than *et al.*, in preparation, Than Than 1976) demonstrate decreased prolyl hydroxylase activity in the skin of jaundiced rats and that this can be correlated with the tensile strength of the wounds in such animals. These observations accord with those of Lee (1972), who demonstrated delay in the migration of fibroblasts and granuloma formation in rats with obstructive jaundice, and those of Bayer and Ellis (1976) which demonstrated delayed wound healing in gastric incisions and parietal peritoneal defects in jaundiced rats.

References

- Bayer, I., and Ellis, H. (1976). Jaundice and wound healing: an experimental study. *British Journal of Surgery*, **63**, 392-396.
- Campbell, R. C. (1974). *Statistics for Biologists*, 2nd edition, pp. 59-61. Cambridge University Press, London.
- Hutton, J. J., Jr., Tappel, A. L., and Udenfriend, S. (1966). A rapid assay for collagen proline hydroxylase. *Analytical Biochemistry*, **16**, 384-394.
- Jiminez, S., Harsch, M., and Rosenbloom, J. (1973).

- Hydroxyproline stabilizes the triple helix of chick tendon collagen. *Biochemical and Biophysical Research Communications*, **52**, 106-114.
- Lee, E. (1972). The effect of obstructive jaundice on the migration of reticulo-endothelial cells and fibroblasts into early experimental granulomata. *British Journal of Surgery*, **59**, 875-877.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**, 265-275.
- McGee, J. O'D., Patrick, R. S., Rodger, M. C., and Luty, C. M. (1974). Collagen proline hydroxylase activity and ³⁵S sulphate uptake in human liver biopsies. *Gut*, **15**, 260-267.
- Than Than (1976). Skin prolyl hydroxylase and tensiometry in jaundice. Thesis, University of Glasgow.
- Than Than, McGee, J. O'D., Sokhi, G. S., Patrick, R. S., and Blumgart, L. H. (1974). Skin prolyl hydroxylase in patients with obstructive jaundice. *Lancet*, **2**, 807-808.
- Torrance, H. B. (1972). In *International Symposium on Sutures in Wound Repair*, p. 41.

The October 1977 Issue

THE OCTOBER 1977 ISSUE CONTAINS THE FOLLOWING PAPERS

- Feulgen microdensitometry and analysis of S-phase cells in cervical tumour biopsies B. DIXON AND R. H. STEAD
- IgA localisation in glomerular diseases W. LAWLER, GEORGE WILLIAMS, P. TARPEY, E. JOAN ACHESON, AND N. P. MALLICK
- Occurrence of e antigen in acute hepatitis B PATRICIA E. GIBSON AND KOKILA RUPARELIA
- Pregnancy and antibody to factor VIII JENNIFER VOKE AND ELIZABETH LETSKY
- Interrelationship of *Chlamydia trachomatis* and other pathogens in the female genital tract G. L. RIDGWAY AND J. D. ORIEL
- Streptococcus milleri* in the appendix PAULINE M. POOLE AND GEORGE WILSON
- Klebsiella: Taxonomy, nomenclature, and communication J. G. BARR
- Incidence of K1 antigen in *Escherichia coli* isolated from blood and cerebrospinal fluid of patients in the United Kingdom T. CHEASTY, R. J. GROSS, AND B. ROWE
- Astrovirus associated gastroenteritis in a children's ward J. B. KURTZ, T. W. LEE, AND D. PICKERING
- Prolonged erythrocyte T-polyagglutination in two children with bowel disorders DAISY OBEID, G. W. G. BIRD, AND JUNE WINGHAM
- Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, WC1H 9JR, price £3.00, including postage
- Formaldehyde induced anti-N: a possible cause of renal graft failure D. W. GORST, R. A. RICHES, AND P. H. RENTON
- Effect of renal transplantation on marrow mast cell hyperplasia of chronic renal failure H. A. ELLIS, KATHLEEN M. PEART, AND A. M. PIERIDES
- Parathyroid carcinoma in familial hyperparathyroidism J. S. DINNEN, R. H. GREENWOOD, J. H. JONES, D. A. WALKER, AND E. D. WILLIAMS
- Dermatitis herpetiformis: a comparative assessment of skin and bowel abnormality T. COONEY, C. T. DOYLE, D. BUCKLEY, AND M. J. WHELTON
- Surface marker and other characteristics of Gaucher's cells G. F. BURNS, J. C. CAWLEY, R. J. FLEMENS, K. E. HIGGY, C. P. WORMAN, C. R. BARKER, B. E. ROBERTS, AND F. G. J. HAYHOE
- Technical methods*
A 'dip-slide' method of sampling bacteria for measuring the efficacy of antiseptics used in labour M. R. WEBSTER
- Identification of non-sporing anaerobic bacteria D. A. LEIGH AND K. SIMMONS
- Virus titrations using trypsin modified erythrocytes K. F. SHORTRIDGE AND L. Y. HU
- Book reviews