Put $k+1 = m$ some odd integer, then
$k = m-1$, Hence we have

$$[A]^m = [A] + \sum_{r=0}^{(m-3)/2} [(p-1)^{2r+1}(p-2)],$$

Case 2.—The Identity was seen to be true for 1 and 3. Let the Identity be true for some odd integer K^1, then K^1+1 will be an even integer. now we must obtain the other form of Identity which is true for even integer.
We have for $n = K^1$ an odd number

$$[A]^{k^1} = [A] + \sum_{r=0}^{(n-1)/2} [(p-1)^{2r+1}(p-2)]_{rep}.$$

Now

$$[A]^{k^1+1} = [A]\,[A]^{k^1}$$

$$[A]^{k^1+1} = [A]\,[A] + \sum_{r=0}^{(n-3)/2} [A]\,[(p-1)^{2r+1} \times (p-2)]_{pep}$$

$$[A]^2 = [I] + [(p-1)^0(p-2)]$$

$$[A]\,[(p-1)^{2r+1}(p-2)] = [(p-1)^{2r+2}(p-2)]$$

$$[A]^{k^1+1} = I_{pep} + [(p-1)^0(p-2)] + \sum_{r=0}^{(k-3)/2} [(p-1)^{2r+2}(p-2)]_{pep}$$

K^1+1 is an even number say n.
then

$$[A]^n = I_{pep} + \sum_{r=0}^{((K^1-1)/2+1)} [(p-1)^{2r}(p-2)]$$

$$[A]^n = I_{pep} + \sum_{r=0}^{(n-2)/2} [(p-1)^{2r}(p-2)]_{pep}$$

Hence the proof :

The Identity can also be proved for even number and odd numbers separately by considering the respective cases for numbers like n and $n+2$. It may be noted that the above proved Identity helps one to calculate the (ij) element $a_{ij}^{(n)}$ of A^n, which represents the number of walks of length n from i to j.

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THE PHYSIOLOGICAL SIGNIFICANCE OF THE INTERACTION OF BILIRUBIN WITH RECONSTITUTED COLLAGEN FIBRILS*

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THE extensive yellowing of the body surface in hyperbilirubinaemic new borns and the restoration to the normal colour of the skin after recovery from jaundice suggest that skin takes active part in the homeostasis of bilirubin at least during the diseased condition. Evidence has been adduced earlier to show that skin epithelium and skin strips of the mouse, rat, guinea pig and man possess a mechanism to accumulate and release bilirubin[1,2]. When skin strips saturated with bilirubin are exposed to light (80 watt) in Krebs Ringer buffer, there is a rapid bleaching of the skin accompanied by the release of water soluble and non-diazotizable degradation products of bilirubin[3].

The uptake of bilirubin by skin is sensitive to temperature indicating that binding of bilirubin to skin strips involves participation of collagen. Results of studies on the binding of bilirubin to collagen fibrils are reported now which suggest that the interaction between collagen and bilirubin may be involved in the uptake of bilirubin by skin.

Collagen was prepared from rat tail tendons according to Glimcher and Krane[4] and purified by dialysis against 0·02 M Na_2HPO_4. The precipitate obtained was redissolved in 1% acetic acid and stored as a lyophilized material. It contained 13·5% hydroxyproline[5]. When required it was dissolved in 100 mM acetic acid and converted to the reconstituted fibrillar form by dialysing at 2° against 200 mM Tris HCl buffer pH 8·6 or 7·5 as required following essentially the procedure of Gross and Krick[6]. The interaction of bilirubin with collagen fibrillar aggregate was followed in 5 ml 100 mM Tris HCl buffer pH 8·6 or 7·5 at 37° C in a two phase system where collagen was present as an opaque rigid gel composed of striated fibrils and bilirubin was in aqueous solution. Different amounts of serum albumin or competing anions could be added to this system as desired. The collagen fibrils were recovered by centrifugation and washed repeatedly with 100 mM Tris HCl buffer and the washed fibrils extracted

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with a solvent system made up of acetone : ethanol : water : acetic acid (v/v, 7 : 7 : 4 : 2) and bilirubin estimated in the extract according to Van Roy *et al.*[7].

As shown in Fig. 1 the interaction of bilirubin with collagen fibrils was affected by concentration of bilirubin in the incubation medium. Binding of bilirubin to collagen fibrils was found to be a linear function of its concentration upto 340 μM reaching saturation at 425 μM of bilirubin and the binding was complete within 60 min. At temperatures below 5° C, binding was depressed and uptake was altogether abolished by heat denaturation of collagen fibrils. The amount of bilirubin bound with unheated control collagen fibrils was 380 *n* mol/mg collagen fibrils whereas after heat denaturation at 52–53° C ftr 1 hr collagen fibrils showed negligible binding affinity for bilirubin.

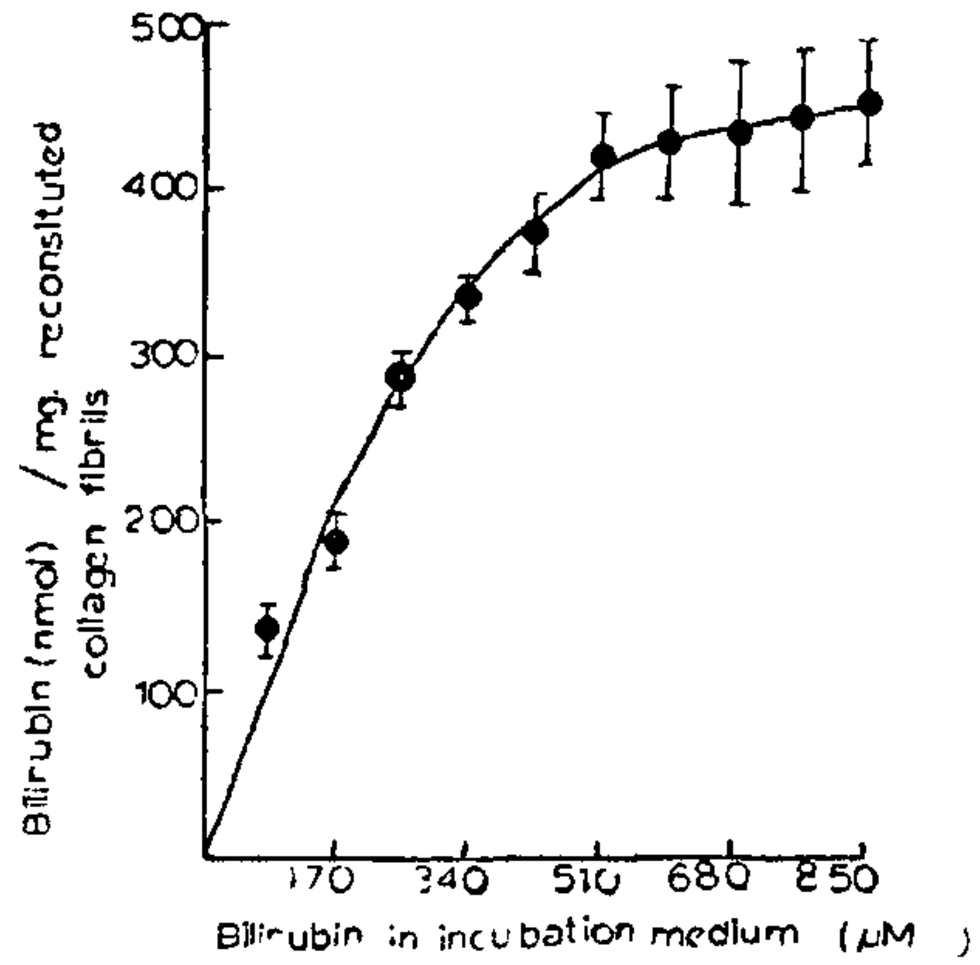

FIG. 1. Binding of bilirubin to collagen fibrils as affected by concentration of bilirubin. Collagen fibrils (0·5 mg) were incubated in 5 ml 100 mM Tris HCl buffer (pH 8·6) containing increasing 85–850 μM concentration of bilirubin at 37° C for 1 h. The binding of bilirubin with collagen fibrils was estimated as described in text. The vertical bars represent the S.D. of the mean value from three separate experiments.

By forming a characteristic bilirubin : albumin complex serum albumin is known to reverse the binding of bilirubin to lipids. The binding affinity of collagen fibrils for bilirubin was, therefore, investigated in different molar concentrations of human serum albumin. Human serum albumin in a concentration of 40–80 μM dissociated only 50% of bilirubin from collagen fibrils : bilirubin complex as shown in Table I.

Salicylate or sulfonamide used in the treatment of neonates does not affect the binding of bilirubin to collagen fibrils. These drugs readily dissociate

TABLE I

Effect of human serum albumin on the dissociation of bilirubin from bilirubin : collagen fibril complex

Bilirubin : collagen fibrils complex were prepared by incubating collagen fibril (0·5 mg), in 5 ml 100 mM Tris HCl buffer (pH 8·6) containing 425 μM bilirubin at 37° C for 1 h, washed free from medium as described in the text. The fibrils were then incubated, in 5 ml Tris HCl buffer (pH 8·6) in presence of 40–80 μM of human serum albumin (HSA). The dissociation of bilirubin was followed for 4 h. Each result in the mean of the values from two separate experiments. Initial bilirubin content was 360 *n* mol/mg collagen fibrils.

Time (h)	Bilirubin (*n* mol) dissociated/mg of collagin fibiils in presence of	
	40 μM HSA	80 μM HSA
0	..	..
2	144	150
4	162	180

the bilirubin from serum albumin : bilirubin complex[9] and increases the incidente of kernicterus[10].

Urea (8·5 M) is known to alter the cross-linking property of collagen fibrils. It was of interest, therefore, to investigate the effect of urea in the binding of bilirubin to collagen fibrils. The results presented in Table II show that urea at 4 M concentrations in the incubation medium diminished 70% of the binding of bilirubin to collagen fibrils.

TABLE II

Effect of urea on the binding of bilirubin with reconstituted collagen fibrils

Collagen fibrils (0·5 mg) were incubated in 5 ml 100 mM Tris HCl buffer (pH 8·6) containing 425 μM bilirubin and increasing concentration of urea at 37° C for 1 h in a metabolic shaker and binding of bilirubin with collagen fibrils was estimated as described in the text. Each result is the mean of the value from two separate experiments.

Urea concentration (M)	Bilirubin (*n* mol) /mg collagen fibrils
None	386
1	256
2	192
3	146
4	102

Urea denaturation and thermal denaturation studies throw light on the importance and organization of the helical structure of collagen fibrils on its affinity for bilirubin. These observations may be of physiological significance in view of the fact that skin provides a semi-solid matrix for the connective tissue. The importance of the role of collagen fibrils is further strengthened by the observation on the lack of affinity of gelatin for bilirubin under identical conditions.

The liver conjugate being still in a developing state may not be able to cope up with all the bilirubin encountered in neonatal jaundice. In such a situation it is presumable that the skin takes the main load of free bilirubin employing native collagen as the binding agent. Collagen thus provides a matrix for the photooxidation of bilirubin to relatively polar degradation products which are then eliminated by the kidney.

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ECOLOGICAL IMPLICATIONS OF HAEMOLYMPH PROTEIN PATTERNS IN SOME AMPHIPOD AND ISOPOD SPECIES

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AMONG the numerous proteins that are to be found in the crustacean haemolymph, two particular components have been easily recognized, when present, owing to their obvious properties—the respiratory pigments and the coagulable proteins. The chemical nature and physical properties of both these proteins have been recently reviewed by Jenuiaux[6], Redmond[9] and by Grégoire[5].

The remaining protein fraction has been resolved into a series of components with different electrophoretic properties[1,2,4,7,11,12]. Although the nature of these proteins is poorly understood, their electrophoretic mobilities in some cases have been used tentatively in taxonomic studies[7].

The aim of the present investigation is to define some of the characteristics of haemocyanin and other blood proteins in a few marine, freshwater and terrestrial isopods, and to determine if the haemolymph protein electrophoretic pattern in these

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