animals in which the vagina opened on day 32, assumed to be extreme lower time limit characteristic of our strain, or before that day, were considered to show an advanced opening of the vagina.

Results. As can be seen from the figure, it is clear that advanced opening of the vagina, appearing after the exposure of F_2 embryos to X-rays, has proved to be (figure, E_2) and inheritable phenomenon which for 7 subsequent generations, so far studied, showed no signs of even gradual return to the state characteristic of normal animals. After the 3rd exposure (figure, E_3) the mean values for opening of the vagina shifted for a 2nd time in the same direction to a new position and the precocity reached what appeared to be its extreme time limit, i.e., day 19, recorded in 2 animals. At the same time, cases of extreme precocity of opening of the vagina (days 22, 21, 20, 19) increased in number. The mean age at vaginal opening of 540 normal rats, recorded in our stock over a period of the last 6 years, was found to be 41.5 ± 0.9 days, ranging from 33 to 66 days.

The b.wt of rats in which the vagina opened precociously, as would be expected, was below normal, but with aging it reached its normal value. It is worth noting that repeated exposure to X-rays on the whole in no way affected the reproductive capacity of animals with precocity or their litter mates (table).

Mean values for the first litter size of animals belonging to all filial generations

Vaginal opening	No. of rats	Litter size (mean ± SE)	Range
Advanced	114	7.0 ± 0.2	2–14
Littermates within the normal values	122	7.0 ± 0.2	2–13
Controls	620	7.0 ± 0.1	2–12

Purification and immobilization of human carbonic anhydrase B by using polyacrylamide gel

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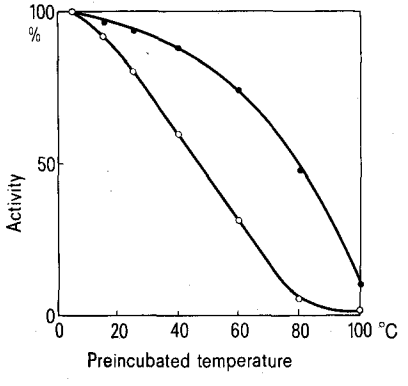
Summary. Human erythrocyte carbonic anhydrase B was purified and immobilized in polyacrylamide gel. As compared to the soluble enzyme, the immobilized enzyme was considerably more resistant to heat and sulphanilamide action.

Recently the study of the theoretical and practical aspects of matrix bound enzymes has gained a lot of interest[2–4]. A number of enzymes and isoenzymes have been purified by biospecific affinity chromatography by using different types of matrixes[3]. Because of the non-availability of preexisting functional groups, the use of polyacrylamide gel matrix has been limited[5]. Falkbring et al.[6] purified carbonic anhydrase B and C by using sulphanylamide-linked Sepharose as the specific matrix. It is known that the rate of the catalytic reaction of erythrocyte carbonic anhydrase is high after isolation from the cells. Chang first noted that, after encapsulation, the enzyme in nylon microcapsules the rate can be maintained as high as in the intact cells[7]. The present investigation deals with the affinity chromatographic purification and immobilization of carbonic anhydrase B by using polyacrylamide gel.



Effect of heat on soluble and immobilized carbonic anhydrase B activity. The soluble and immobilized enzymes were preincubated at different temperatures for 90 min prior to incubation at 25 °C for 10 min. Enzymatic activity was expressed as percentage of the respective controls (5 °C preincubation for 90 min). ○—○ soluble enzyme; ●—● immobilized enzyme.

Materials and methods. P-Nitrophenylacetate was prepared by acetylation with acetic anhydride in pyridine and recrystalized from dilute alcohol[8]. Polyacrylamide gel beads were prepared[9], pulverized and 100–200 mesh sieved materials were used as the matrix for affinity chromatography. The beads were activated to acyl azide derivative and coupled with sulphanilamide at 0–5 °C for 30 min. Partially purified enzyme was obtained by ethanol-chloroform extraction method[8] and dialyzed against 0.1 M Tris-sulphate buffer, pH 7.5, prior to couple with sulphanilamide-linked polyacrylamide gel. The coupling was conducted at 20 °C for 0.5 h and washed with the same buffer until no protein was detected in the washings. Carbonic anhydrase B was eluted with buffer containing 0.2 M KI and dialyzed against 0.05 M Tris-sulphate buffer, pH 7.5. In all these cases, soluble protein was estimated according to Lowry et al.[11]

1 Acknowledgments. I thank Prof. R. L. Brahmachary for providing me all the laboratory facilities to carry out the investigation.

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The purified carbonic anhydrase B was immobilized in polyacrylamide gel by entrapment process[12]. The enzyme was mixed with varying amounts of monomer, containing 5% cross linking material, in 0.1 M Tris-sulphate buffer, pH 7.5. Immediately after addition of the catalyst mixture and enzyme protector (N,N,N′,N′-tetramethylethylenediamine, 0.005 ml/ml of gel; ammonium persulphate, 3 mg/ml of gel; cysteine and histidine 1 mg each/ml of gel, which act as the protector of the enzyme during polymerization; dissolved in 2.0 ml of the buffer) the solution was poured into the reaction vessel and polymerization was carried out under fluorescent light for 30 min at 0–5 °C. After polymerization the polymer was crushed, washed with the same buffer and dried in the cold. Protein content of the enzyme beads was estimated according to Spackman et al.[13]. The assay of the enzyme was performed by determining its esterase activity using p-nitrophenylacetate as the substrate[14]. Thermostability of the immobilized enzyme was tested as follows: Samples of soluble and immobilized enzymes were preincubated at different temperatures from 5 °C to 80 °C for 90 min prior to incubation with p-nitrophenylacetate at 25 °C for 10 min.

Results and discussions. Carbonic anhydrase B may readily be purified by using sulphanilamide-linked polyacrylamide gel. The purification of the enzyme was noted by comparing the specific activity of the enzyme before and after chromatographic purification. The specific activity was increased from 1.2 U/mg protein to 18 U/mg protein. The table illustrates the optimum concentration of the polymer (20%) for the entrapment process. At this concentration of the gel, the 'enzyme beads' show optimum activity of the enzyme. Part of this activity may be lost in the process of polymerization. The recovery of some of the activity was found after addition of cysteine and histidine at the time of polymerization. It is known from the 3 dimensional structure of the enzyme that a number of histidine molecules, in the surroundings of the zinc ion of the active centre, play an important role in its activity[15]. It may so happen that the externally added histidine protects the free radical damage of the enzyme-histidine in the process of polymerization. The values of Km (5.9 mM for soluble and 5.6 mM for immobilized enzyme) and of pH optimum (8.0 for soluble and 7.7 for immobilized enzyme) were comparable. These suggest that the affinity to the substrate may not be affected by the entrapment process. It was found that 2×10^{-5} M sulphanilamide, a potent inhibitor of the enzyme, had no inhibitory effect on the immobilized enzyme, but 50% of the activity of the soluble enzyme was destroyed at the same concentration of the inhibitor. At the concentration of 5×10^{-4} M sulphanilamide, the activity of the soluble enzyme was completely destroyed, but the immobilized enzyme still showed 40% of the activity. The figure illustrates that about 50% of the activity of the immobilized enzyme was maintained even after preincubation at 80 °C for 90 min, while under the same condition no activity of the soluble enzyme was found. Thus the entrapment process in polyacrylamide gel may protect the active site of the carbonic anhydrase B from the effects of temperature and sulphanilamide action.

Activity and extent of entrapment of carbonic anhydrase B in polyacrylamide gel (average of 2 separate preparations)

Polymer (dry wt/100 ml of wet gel)	Added enzyme (mg/g of gel)	Protein content (mg/g of dry gel)	Bound enzyme (%)	Activity (U/g of gel)	Activity (U/mg of bound protein)
15	6.65	4.25	64	35.3	8.3
20	6.25	4.05	77	43.8	10.8
25	4.27	2.95	69	24.1	8.2

1 unit (U) is expressed as μmole of p-nitrophenol liberated per min at 25 °C.

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Studies on peripheral neurons and neurohaemal tissue in the thorax of the stick insect (*Carausius morosus*)[1]

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Summary. The distribution of peripheral neurosecretory and non-neurosecretory neurons, and transverse nerve neurohaemal swellings, is described in the thorax of *Carausius morosus*. The electrical activity of the isolated transverse nerves has been recorded.

The presence of neurosecretory and non-neurosecretory neurons lying on peripheral nerves in insects has now been described in several orders[2–7]. The most extensive study of this system has been made in the abdominal segments of the stick insect, *Carausius morosus*[2,3]. In this insect the 2 types of cells have a very different morphology and ultrastructure, and consequently it is easy to distinguish between them using methylene blue stain[3]. In this paper we describe the presence of both neurosecretory and non-neurosecretory neurons lying on major nerves in the thorax of the stick insect, as well as neurohaemal swellings which occur on the lateral branches of the median nervous system. Finlayson and Osborne[8] have shown that spontaneously generated action potentials can be recorded from the isolated neurohaemal tissue on the abdominal transverse nerves, and they have suggested