Showing the level of 5 HT in different organs following electric shock (μg/g of tissue)

Tissues	Control ± SD	1st week ± SD	2nd week ± SD	3rd week ± SD	4th week ± SD
Heart	0.721 ± 0.197	1.644 ± 0.097***	3.345 ± 0.344***	2.210 ± 0.102***	1.863 ± 0.061***
Whole brain	0.591 ± 0.148	2.443 ± 0.103***	1.171 ± 0.026***	1.571 ± 0.066***	0.975 ± 0.045
Kidney	2.317 ± 0.865	1.227 ± 0.188*	2.760 ± 0.187	3.020 ± 0.120	2.334 ± 0.026
Liver	1.953 ± 0.607	1.900 ± 0.290	1.990 ± 0.130	2.819 ± 0.148**	1.470 ± 0.367

*$p < 0.025$, significant, **$p < 0.01$, significant, ***$p < 0.001$, significant (compared to control).

Material and methods. 30 healthy male albino rats weighing between 100 and 125 g were selected, out of which 6 were kept under normal laboratory conditions to serve as a control group. The other 24 rats were subjected to electric shock through their feet[11] (an intermitant current of 60 volts daily for 30 min for 4 weeks). The animals were sacrificed in different weeks (6 in each week) and the tissues were collected in ice-cold perchloric acid (0.4 N PCA) for 5-HT estimation following the method of Snyder et al.[12] within 48 h of sacrifice. The data were analyzed with the Student t-test.

Results. The normal tissue levels of 5-HT have been shown in the table. After electric shock, the level of 5-HT in brain and heart significantly increases in different weeks, but the highest level was observed in the 1st week in brain and in the 2nd week in the heart. In 4th week the level remains still significantly high. The levels of 5-HT in kidneys and liver were found highest after 3rd week. The kidneys show a significant fall after 1st week, followed by a significant increase after 2nd and 3rd week. At the end of 4th week, the level becomes normal. There was no significant change in case of liver after 1st and 2nd weeks. At the end of 4th week the level comes down below the normal level (statistically insignificant) (table).

Discussion. In response to electric shock, the rats showed significantly elevated levels of 5-HT in different tissues. The variations of the 5-HT content in different tissues of the normal subjects may be due to the number of enterochromaffin cells and the serotonergic fibres present in a particular tissue. It may also be due to the tolerance of 5-HT by the tissue, because 5-HT is a vasoconstrictor substance, the presence of which in a higher or lower concentration may be responsible for the maintenance of the normal microcirculation. Thus, following stress, the degree of change in the level of 5-HT content in different tissues is not uniform. Hence the physiological responsiveness of the particular tissue to a particular stimulus appears to be responsible for such variations.

In case of liver, heart, kidneys and brain tissues, it is obvious that the animals may adapt the normal physiology after repeated stress. The disturbances and the mechanism by which the level of 5-HT increases following stress, has been demonstrated by many workers[13–15]. The study of disturbances caused by stress may be helpful in understanding the pathophysiology of various stress disorders.

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Nuclease for DNA apurinic sites in chicken liver

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Summary. Chicken liver crude extract produced acid-soluble diphenylamine-positive material in the presence of depurinated and alkylated DNA, while the formation of such material from normal and single stranded DNA was comparatively low. The maximum acid-soluble material produced was not increased further by alkali, indicating that the enzymatic action is mostly directed towards apurinic sites.

Depurination of DNA and its repair has been the subject of some interest in the recent past[2–6]. It has been suggested that the cellular DNA may be spontaneously undergoing some depurination under physiological conditions[3]. Endonucleases specific for apurinic sites that may have a role in the repair of such sites, have been shown to exist in bacteria and mammalian cells[2,4–6]. In this communication we report the presence of a nuclease (s) that degrades alkylated and depurinated DNA in chicken liver.

Materials and methods. The method for preparation of depurinated DNA was essentially that of Hadi and Goldthwait[2] with some modifications[7], and it involved heating the DNA at pH 3.5. The preparation of alkylated DNA and depurinated DNA from alkylated DNA has been described[7,8]. Chicken liver crude extract was prepared by homogenizing freshly excised liver with 3 volumes of cold tris-HCl buffer (0.05 M, pH 7.0). The homogenate was centrifuged at 1000 × g for 5 min and the supernatant obtained used as the source of enzyme. Nuclei were isolated and purified by a standard procedure[9]. The procedure for extraction of enzyme from nuclei was essentially as described by Ohtsuka et al.[10].

Results and discussion. Figure 1 shows the effect of chicken liver crude extract on native and depurinated DNA. With each substrate, the perchloric acid soluble

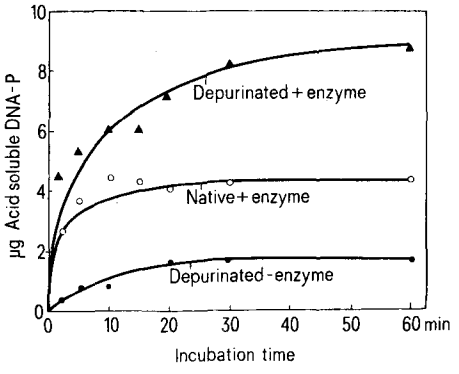

Fig. 1. Effect of crude extract upon normal and depurinated DNA. The reaction mixture in 1 ml contained 650 µg of substrate (depurinated or native DNA), 1 mM $MgCl_2$, 1 mM 2-mercaptoethanol, 0.08 ml tris-HCl buffer (0.5 M, pH 7.5) and 0.43 mg of enzym eprotein. The incubation was carried out at 37 °C and aliquots of 1 ml were removed at various time intervals and added to 10 ml centrifuge tubes containing 0.2 ml of bovine serum albumin (2 mg). The tubes were shaken well and reaction stopped by adding 1.2 ml of cold 14% perchloric acid. After 1 h at 4 °C the acid-insoluble material was centrifuged off and DNA phosphorus (DNA-P) in the supernatant was estimated by the procedure described by Schneider[11].

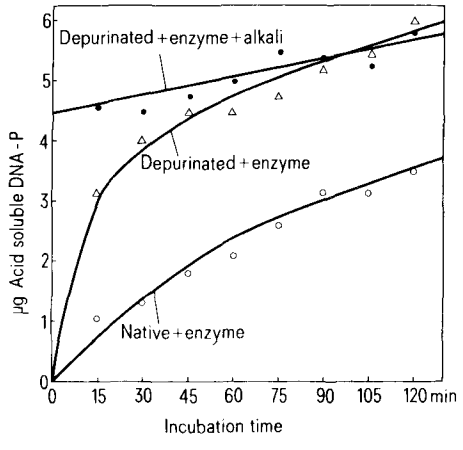

Fig. 2. Action of nuclease on apurinic sites in DNA. The reaction mixture contained per ml 500 µg of substrate (normal and depurinated DNA) and 0.38 mg of enzyme protein. 1 ml aliquots from depurinated DNA reaction mixture were removed at various time intervals and incubated further with 0.2 ml of 2 N NaOH for 30 min at room temperature before stopping the reaction.

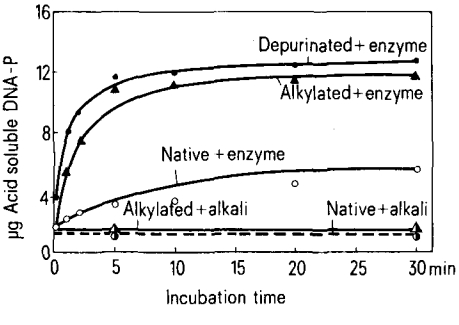

Fig. 3. Effect of crude extract upon normal, alkylated and depurinated DNA. DNA was alkylated and depurinated DNA obtained from it as described in the methods. The reaction mixture per ml contained 730 µg of substrate and 0.42 mg of enzyme protein. The other details were as described for figure 1.

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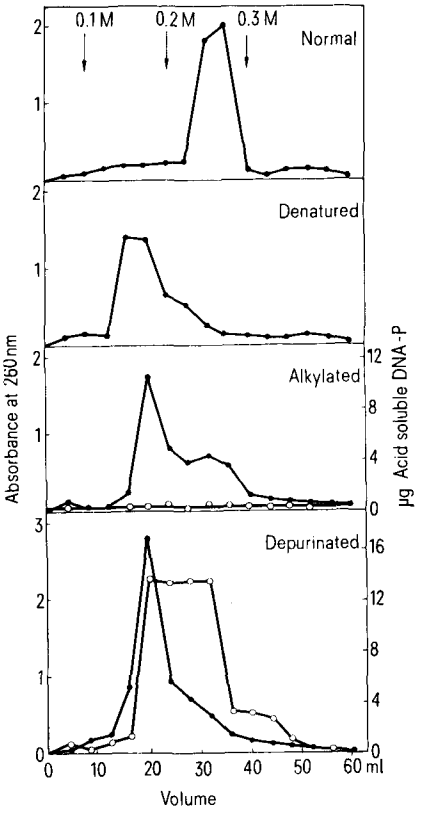

Fig. 4. Hydroxylapatite chromatography of alkylated and depurinated DNA. 2 mg of DNA in 8 ml of TNE (0.01 M tris-HCl, 0.01 M NaCl and 2×10^{-4} M EDTA, pH 7.4) was loaded at fraction number zero and immediately followed by elution with sodium phosphate buffers of molarities shown (pH 7.0) containing 1% HCHO. The size of the column was 1×7 cm and 4 ml fractions were collected at the rate of 8 ml/h. Recoveries were: normal DNA 96.5%, denatured DNA 90%, alkylated DNA 93% and depurinated DNA 98%. In the case of alkylated and depurinated DNA, 1 ml aliquots from each fraction were treated with alkali and the non-hydrolyzed nucleic acid precipitated with perchloric acid. Acid-soluble DNA-P was measured in the supernatant as described[11]. Absorbance at 260 mµ (●—●). Acid-soluble DNA-P released on alkaline hydrolysis (○—○).

DNA-phosphorus (DNA-P) was measured after different times of incubation[11]. The release of acid soluble material as a function of time from depurinated DNA is found to be considerably higher than from native DNA. Depurinated DNA incubated without enzyme shows some degradation with time. This could be a tris catalyzed reaction since amine buffers are known to catalyze the degradation of depurinated DNA[12]. Similar results were obtained when the enzyme extracted from purified nuclei was used. The degradation of single stranded DNA containing no apurinic sites was also found to be considerably lower as compared with that of depurinated DNA. In order to demonstrate that the cleavage of depurinated DNA was mostly directed towards apurinic sites, the experiment shown in figure 2 was done. After treatment of depurinated DNA with enzyme for indicated period of time, the reaction mixture was further treated with alkali. This would result in the cleavage of all apurinic sites left undegraded by the enzyme. Addition of NaOH after 90 min of incubation with enzyme did not increase further the acid-soluble material already produced enzymatically. This indicates that the sites of action of alkali were already acted upon by the enzyme. It is, therefore, concluded that the enzyme and alkali cleaved the depurinated DNA at or near apurinic sites.

Depurinated DNA has been shown to be an intermediate in the degradation in vitro of alkylated DNA[13]. Endonucleases specific for alkylated DNA have been reported in B. subtilis[14] and E. coli[15]. DNA was alkylated by dimethyl sulphate, which is a weak carcinogen. Depurinated DNA was obtained by mild heating of alkylated DNA[8], which resulted in the appearance of apurinic sites as shown by alkaline hydrolysis of such DNA. As shown in figure 3, the production of maximum acid-soluble material from alkylated and depurinated DNA is significantly higher than that from native DNA. Alkylated DNA alone in the presence of alkali did not show any degradation. The procedure of heating DNA at pH 3.5 for preparation of depurinated DNA results in the complete denaturation of DNA at 52 °C[2]. In order to determine the native or denatured states of alkylated DNA and depurinated DNA obtained from it, hydroxylapatite chromatography of such DNA was done. The results are shown in figure 4. Whereas part of the molecules of alkylated DNA retain at least partial double strandedness under our conditions, the depurinated DNA is completely denatured. The denaturation of alkylated DNA is a time-dependent phenomenon since it was observed that after 24 h at room temperature this DNA becomes completely denatured.

The apurinic site specific nuclease reported here and by other authors in different systems may be the first enzyme required for the repair of apurinic sites in DNA. This assumes that the mechanism of repair of depurinated DNA is similar to that of UV-irradiated thymine dimer containing DNA. Indeed, using E. coli apurinic site nuclease, Verly et al.[16] have recently demonstrated in vitro repair of apurinic sites in DNA. The nuclease activity on alkylated DNA observed by us raises an interesting question. The enzymatic degradation of alkylated DNA may involve depurination as an intermediate step similar to non-enzymatic degradation of alkylated DNA. A single enzyme may, therefore, be required for both the steps. Conversely 2 separate enzymes may be involved as has been suggested in the case of E. coli endonuclease II[17]. However, the question can only be answered after the enzyme has been purified.

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Serum cholinesterase: Function in lipoprotein metabolism

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Summary. Human serum beta-lipoproteins, isolated by percipitation with heparin-calcium mixture, showed cholinesterase activity. The enzyme activity was almost proportional to the lipoprotein concentration. Rats, treated with neostigmine, a cholinesterase inhibitor, showed a significant decrease in serum beta-lipoprotein and in the incorporation of H^3-lysine into the lipoprotein compared to untreated controls. The decreased incorporation of H^3-lysine into beta-lipoprotein was associated with increased labelling of alpha-lipoprotein. There was no significant difference in the labelling of pre-beta-lipoprotein. We propose that LDL is formed from VLDL in the presence of cholinesterase.

The biological role of serum cholinesterase (ChE) is unknown. However, a relationship between ChE and serum beta-lipoprotein (BLP) has been demonstrated. It has been shown[3] that human BLP released ChE upon ultrasonication and that BLP and ChE recombine upon standing. We demonstrated[4] that ChE inhibitors like physostigmine can destabilize BLP. When BLP was treated with phospholipase D, ChE was released and at the same time, the lipoprotein showed altered electrophoretic mobility[5]. This suggests that the site of attachment of ChE to the lipoprotein molecule is at the phosphorylcholine part of lecithin. It is obvious that a close structural relationship exists between phosphorylcholine and acetylcholine, a natural substrate for ChE.

We next observed[5,6] that when hyper-pre-beta lipoproteinemia was induced in rabbits by treating them with E. coli endotoxin, there was a marked increase in the ChE activity. This activity decreased with the conversion of pre-beta into beta-lipoprotein. This suggests that BLP can bind ChE.

In nephrotic syndrome, an unexplained hypercholesterolemia and hyper-beta-lipoproteinemia has long been re-