in 0·1 M (pH 7·9) phosphate buffer, *N*-acetylserotonin was almost twice as active as serotonin. These observations suggested the presence of a second, more heat-labile, HIOMT-like enzyme.

HIOMT activity was also examined in the pineal glands of a number of adult avian species kept in diurnal lighting (Table 2). With the exception of the pigeon, all the birds studied had as much enzyme activity as mammals or more. In addition, bird pineal extracts *O*-methylated serotonin with varying degrees of effectiveness. HIOMT was also assayed in various brain areas and tissues (eyes, liver, lung, heart and gut) of quail and found to be undetectable. The high activity of HIOMT in bird pineal glands indicates that this species has a considerable capacity to synthesize melatonin. This indole has been found to inhibit gonadal activity in mammals and to lighten the skin of amphibians.[8] Recently it has been reported that small doses of melatonin also inhibit gonad growth in the Japanese quail[9] under certain lighting conditions.

Other *O*- and *N*-methyltransferase enzymes such as histamine-*N*-methyltransferase, catechol-*O*-methyltransferase, and phenyl-ethanolamine-*N*-methyltransferase (the enzyme that *N*-methylates noradrenaline to adrenaline) were also measured in bird pineal glands (Table 2). The pineal glands of quails and leghorn chickens had relatively high histamine-*N*-methyltransferase activity. The quail pineal glands had large amounts of the adrenaline-forming enzyme, even more than the adrenal gland where this enzyme is highly localized.

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Md., U.S.A., and
Department of Zoology,
University of Alberta,
Edmonton, Canada

JULIUS AXELROD
JEAN K. LAUBER

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Interaction with heparin of deoxyribonucleoprotein complex damaged *in vivo* by nitrogen mustards

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THE TREATMENT with biological alkylating agents simulates in many regards the effect of irradiation.[1–3] In experiment *in vitro* they damage DNA and other important biological molecules by alkylating and cross-linking them in various groups.[4, 5] The primary site of reaction[5] responsible for the effect observed *in vivo* remains, however, in spite of intensive study, still obscure.[6]

The treatment of animals with alkylating agents, similarly as irradiation, leads to profound changes in the lymphatic tissues, causing intermitotic death of lymphocytes, followed by rapid cellular depletion of the tissues.[1, 7] Among the first biochemical changes in lymphatic tissues after both treatments is damage to the nucleoprotein complex of cell nuclei which is seen as an increase in the amount of

polydeoxyribonucleotides,[8] i.e. free DNA, released from the deoxyribonucleoprotein complex (DNP) Such changes have been described in previous papers[9–11] following irradiation. Recently another sign of postirradiation damage to the DNP was found, namely increased sensitivity of DNP to the action of various polyanions, such as heparin and dextran sulphate.[12, 13] In the present communication we described the results of similar comparative experiments, dealing with the interaction of heparin with DNP after *in vivo* treatment of mice with nitrogen mustards.

In these experiments random-bred female mice (strain H), aged 6–8 weeks, were used. Two various *N*-mustard type drugs were injected i.p. to mice: TS 160 Spofa (Tris-/2-chlorethyl/amine hydrochloride) in the amount 0·2 mg/10 g of mouse weight and Degranol Chinoin (1,6 *bis*/2-chlorethylamino/1,6 deoxy-D-mannit dihydrochloride) 2 mg/10 g in physiological saline. In both cases the doses exceed the LD_{50}/30 days. Mice were killed by decapitation 8 hr later. Thymuses and spleens were rapidly removed, pooled from several mice and homogenized in cooled 0·14 M NaCl solution with 0·015 M sodium citrate. After centrifugation (2500 *g*, 15 min) the sediment was washed by resuspending it twice in 0·14 M NaCl solution and filtered through layers of silk gauze.

Equal parts of this suspension of crude DNP (containing 250–450 μg of DNA) were incubated for 30 min at 5° in 4 ml of 0·14 M NaCl solution, containing varying amounts (0–250 μg/ml) of heparin (Spofa, 100 i.u./mg). After incubation, the non-attacked DNP was sedimented by centrifugation (2500 *g*, 15 min), DNA released into the supernatant was precipitated by addition of perchloric acid (to the concentration 0·25 N) and in both fractions DNA was estimated by a modified diphenylamine reaction.[8] The amount of DNA released into the solution under the action of heparin was expressed as percents of the total amount of DNA present in the specimen.

The results of our experiments are given in Figs. 1 and 2. It can be seen, that the interaction with heparin of DNP from normal tissues differs essentially from the reaction of DNP from tissues of treated mice. The amount of DNA released from normal DNP increases only slowly on incubation with rising heparin concentrations, reaching 9–12 per cent of the total amount of DNA in the presence

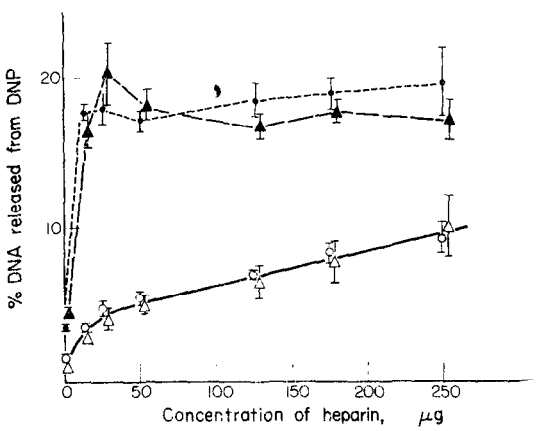

FIG. 1. The release of DNA from the suspension of DNP isolated from spleens of normal mice and mice treated with TS 160 or Degranol on incubation with increasing amounts of heparin. Means and S.D. of the means are shown.

△ From 5 experiments with DNP of normal mice (control groups in TS 160 experiments), ○ from 9 experiments with DNP of normal mice (control groups in Degranol experiments), ▲ from 7–9 experiments with DNP of mice killed 8 hr after TS 160 treatment, ● from 10–12 experiments with DNP of mice killed 8 hr after Degranol treatment.

of 250 μg heparin/ml. In the case of DNP isolated from tissues of mice treated with *N*-mustards, already a small amount of heparin leads to substantial release of DNA into the solution, without any pronounced increase with further rise of heparin concentration.

The maximum response to heparin of spleen DNP from mice treated with both TS 160 and Degranol reaches 20 per cent (Fig. 1), i.e. similar to the level observed in previous experiments after total-body

irradiation.[12] In the interaction of thymus DNP with heparin (Fig. 2) a marked difference can be observed between the effect of both *N*-mustards used. Whilst thymus DNP of TS 160 treated mice releases about 20 per cent of DNA under the incubation with heparin, the response of thymus DNP from Degranol treated mice reaches 40 per cent, i.e. a higher level than was observed in experiments after irradiation.[12]

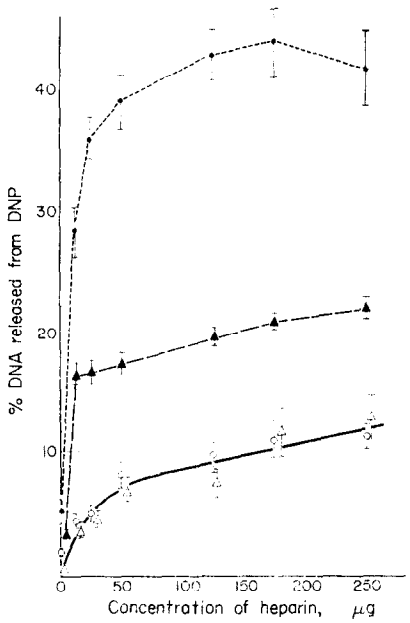

FIG. 2. The release of DNA from the suspension of DNP isolated from thymuses of normal mice and mice treated with TS 160 or Degranol on incubation with increasing amounts of heparin. (Symbols as for Fig. 1.)

The present experiments were performed, as noted, with doses exceeding $LD_{50}/30$ days, i.e. in the region of doses where the maximal response is already obtained, as judged from previous experiments with total-body irradiated animals.[13] Whereas the changes of spleen DNP from irradiated and both TS 160 and Degranol treated mice are almost the same, the sensitivity of thymus cells to various treatments is different. A better insight into the reason of the difference in the response of thymus to various treatments used can be obtained only in further experiments involving other criteria for evaluating cellular damage.

The mode of interaction of DNP with polyanions is not clear and this is one of the reason why we are not able to answer the question, at present, what is the chemical and/or structural base underlying the increased sensitivity of DNP from treated mice to polyanions. Hagen,[14] observing an easier deproteinization of DNP isolated from irradiated rats, considered this change as a sign of a "labilization" of the band between DNA and protein in DNP complex. Real nature of this labilization is, however, unknown.

It follows from these experiments, that the general character of the increased DNP sensitivity to the polyanion parallels the previously observed changes in the release of polydeoxyribonucleotides[13] and is very similar to that, observed after total-body irradiation.[10, 12] It is, however, impossible to give any explanation about the origin and mechanism of the observed damage. The only clear evidence is, that the changes of DNP are related to the early, intermitotic death of lymphocytes,[15] the nature of which is different from the other types of cellular damage caused by radiation and alkylating agents, i.e. from the metabolic impairments and mitotic delay.

Summary—The treatment of mice with two alkylating agents, i.e. Tris-(2-chlorethyl) amine hydrochloride and 1,6 *bis*(2-chlorethyl-amino) 1,6 deoxy-D-mannit dihydrochloride, leads to profound damage to desoxyribonucleoprotein complex in thymus and spleen, manifested in a distinctly increased sensitivity of DNP to the action of heparin.

Institute of Biophysics,
Czechoslovak Academy of Sciences,
Brno

J. MATYÁŠOVÁ
M. SKALKA*

* Present address: Department of Research and Isotopes, IAEA, 1010 Vienna, Austria.

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The non-reactivity of 1,2-fluorenoquinone-2-acetimide with deoxyribonucleic acid and soluble ribonucleic acid*

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IN A STUDY of the relationship of the combination of carcinogens with tissue components to the induction of tumors by these agents, the possibility that 1,2-fluorenoquinone-2-acetimide (FQI) might react with nucleic acids has been examined. FQI, a possible metabolite of the carcinogen 2-acetylaminofluorene, and other *o*-quinone imides combine irreversibly with free amino groups of protein to give stable adducts exhibiting increased absorption in the u.v. region and new absorption maxima in the visible region.[1] In order to see whether similar reactions occur with nucleic acids, soluble RNA and native and heat-denatured DNA were characterized after exposure to FQI under conditions which were shown to give rise to combination of this compound with protein.

DNA from calf thymus (Calbiochem, Los Angeles, Calif.) and soluble RNA from yeast (Calbiochem, Los Angeles, Calif.) were exposed to FQI by adding solutions of the quinone imide in dioxane to buffered solutions of the nucleic acids at room temperature and allowing them to stand overnight at 4°. The nucleic acids were then reisolated from the incubation mixture by successive extractions

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