TECHNICAL PAPERS

A Coagulation Defect Produced by Nitrogen Mustard[1]

T. R. SMITH, L. O. JACOBSON, C. L. SPURR, J. G. ALLEN, and M. H. BLOCK[2]

Departments of Medicine and Surgery, The University of Chicago

The observations of Allen, *et al.* (*1, 2*) have indicated that exposure to ionizing radiations, especially in the lethal or semilethal range, produces a prolongation in the whole-blood coagulation time of humans and animals. This effect was found to be due to the appearance of an anticoagulant in the blood biologically indistinguishable from heparin.

Recent observations in our laboratories on 5 human subjects with neoplastic disease and on normal rabbits (*3*) indicate that one of the nitrogen mustards (methyl-bis(β-chloroethyl)amine hydrochloride) produces a coagulation defect identical to that induced by radiations. Previous reports (*4, 5*) dealt with the serious toxic reactions involving the blood and blood-forming tissue of patients with neoplastic disease treated with this agent. A standard course of treatment[3] was found which was relatively safe in the sense that spontaneous recovery always followed in spite of the occasional severe destructive effects on the hemopoietic system. In no instance was the whole-blood clotting time considered to be significantly prolonged.

Of the 5 patients considered in this report, two were given 0.1 mg/kg of body weight on four consecutive days; the third received four injections of 0.1 mg/kg at intervals of 12 hrs; the fourth was given four injections of 0.1 mg/kg at intervals of 7 hrs; and the fifth patient was given only two injections of 0.3 mg/kg, 6 hrs apart. The total dose in each of the 5 cases was 25.4, 26.8, 22.4, 20.0, and 38.0, respectively. Within two weeks after administration of the drug, all 5 patients developed a moderate anemia, severe leucopenia, thrombocytopenia, prolonged bleeding time, cutaneous petechiae, and ecchymoses. The whole-blood coagulation time (Lee White) in each instance was definitely prolonged or was incoagulable in 24 hrs. Gastrointestinal and cerebral hemorrhages were serious complications, and death occurred in two patients. The intravenous administration of toluidine blue or protamine at a dose of approximately 2 mg/kg was usually sufficient to reverse the clotting time to normal for a 24-hr period. The initial or a higher dose of the antiheparin substance was repeated each 24 hrs, as indicated, in an attempt to maintain a normal clotting time. In the test tube also, prolonged whole-blood clotting time could be returned to normal limits by the addition of protamine or toluidine blue. The prothrombin time of all 5 of the individuals was normal.

This report is intended to emphasize the fact that in certain patients nitrogen mustard therapy may produce serious or even fatal complications as the result of the presence of an anticoagulant (heparin ?) in the blood. This hemorrhagic effect can be neutralized *in vivo* and *in vitro* by specific antiheparin substances such as protamine, toluidine blue, and other thionine dyes. This observation is of further interest in drawing attention to the similarities in the biological effects of ionizing radiations and nitrogen mustards.

[1] Supported in part by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

[2] Senior Research Fellow (Cancer), U. S. Public Health Service.

[3] One-tenth mg/kg of body weight given daily for four days.

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Recovery of Pneumoencephalitis (Newcastle) Virus From the Air of Poultry Houses Containing Infected Birds

P. D. DELAY, K. B. DEOME, and R. A. BANKOWSKI

Department of Veterinary Science, University of California, Berkeley

This report describes the recovery of pneumoencephalitis virus from air contaminated as a result of natural infection. The viruses of poliomyelitis (*3*), influenza (*4*), and laryngotracheitis (*1*) have been transmitted by the exposure of susceptible animals to artificially contaminated air. It was assumed, therefore, that these and possibly other virus diseases may be transmitted by the air-borne route under conditions of natural infection, but the presence of virus in such air had not been demonstrated. In the case of pneumoencephalitis, field observations and laboratory experiments indicated the probable transmission of this infection by the air-borne route; hence, these experiments were undertaken to demonstrate

the infectivity of air samples collected from houses containing birds infected with this disease.

The air samples were collected from poultry houses located in an area where pneumoencephalitis vaccination trials had been conducted for several years. Each house contained one pen of nonvaccinated control birds and four pens of birds which had been vaccinated with formalized pneumoencephalitis vaccine. Air samples were taken from the control pens of houses #6 and #9. The control pen in house 6 contained 280 birds which had shown the first clinical evidence of pneumoencephalitis 7 days before the air was sampled; that in house 9 contained 335 birds which had shown the first evidence of infection 10 days prior to the time when the air was sampled. The houses were of shed-roof-type construction containing 5 pens $18' \times 32'$. Each pen had four open windows across the front and two ventilators in the rear. The air in these pens contained a considerable amount of suspended dust, but the dust concentration probably was not different from that usually encountered in poultry houses where deep litter is used.

The air samples were drawn through allantoic fluids harvested from normal 10-day chick embryos by means of the sampling atomizers previously described by DeOme *et al.* (*2*). The air inlets of the atomizers were adjusted to the level of the birds' heads. The volumes of air sampled in houses 6 and 9 were 540 liters and 1,080 liters, respectively. The allantoic fluids from the atomizers were frozen in dry ice immediately after the air was drawn through them, returned to the laboratory, thawed, treated with 10,000 units of penicillin and 24,000 of streptomycin/ml of inoculum, and incubated for 4 hrs at 9° C.

Subsequent culture tests indicated that the treated fluids contained no viable bacteria. Thirteen of fifteen 11-day-old chick embryos injected with 0.2-ml portions of the sample obtained in house 6 died at a mean age of 6.5 days. In two subsequent embryo passages, one-half of generation 2 embryos died at a mean age of 3.6 days, and all of generation 3 embryos died at a mean age of 4 days. The sample from house 9, when inoculated into chick embryos, yielded similar results. The allantoic fluids collected from embryos of generation 3 agglutinated chicken red blood cells, whereas those of generations 1 and 2 failed to do so. Chickens inoculated with the allantoic fluids from embryos of generation 3 developed symptoms of pneumoencephalitis and, upon postmortem examination, presented marked lesions typical of those produced by cultured pneumoencephalitis virus.

These data show that air from an infected house, sampled in 540- or 1,080-liter quantities, contained virus in sufficient concentrations to infect chick embryos.

In an attempt to test the infectivity of such air for chickens, four normal chicks were exposed to the aerial environment of a house containing birds affected with pneumoencephalitis. The four chicks were confined in wire cages suspended $4\frac{1}{2}'$ above the floor. Care was taken to prevent contact with contaminated material which was not air-borne. Respiratory symptoms were observed on the 6th day. On the 8th day the sera from three of the four birds were found to contain hemagglutination-inhibiting antibodies. On the 15th day the birds were returned to the laboratory and challenged with 2×10^5 chicken mid of pneumoencephalitis virus. All four birds were refractive.

No previous report of the isolation of virus from air contaminated as the result of natural infection has been found in the literature.

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High-Level Gravels of Western Grand Canyon

Donaldson Koons

Department of Geology,
Colby College, Waterville, Maine

Field studies[1] conducted during the summer of 1946 in the vicinity of Western Grand Canyon, Arizona, and the Hualpai Indian Reservation disclosed the presence of large areas of gravel composed of pebbles foreign to the region. Deposits are found at heights of 3,500–4,000′ above present river level. An occurrence of these gravels near Frazier Well, in the Hualpai Reservation, is mentioned by Darton (*1*) without discussion, and two small areas are indicated on the geological map of Arizona (*2*).

Detailed study of the gravel deposits showed that they were more extensive than had been indicated previously and that two types of gravel could be distinguished. The first is composed of poorly rounded and sorted pebbles of limestone, sandstone, and chert of local derivation and rests on steep bed-rock slopes. It is interpreted as a talus and alluvial cone deposit preserved in favorable locations during reduction of the cliffs at the bases of which it was deposited. The name 'Robbers Roost Gravel' is proposed for this group, because of its occurrence in good exposure near the mesa of the same name.

The second gravel, more extensively developed, consists of well-rounded pebbles and boulders of vein quartz, granite, gneiss, schist, red and white quartzite, and sandstone. These gravels are best exposed near Frazier Well and the name 'Frazier Well Gravel' is consequently proposed. The deposits vary in thickness from a thin veneer to more than 200′, the maximum thickness being found near Frazier Well. Present elevation of the deposits varies from 5,660′, 12 miles southwest of Frazier Well to 7,150′, 5 miles northwest of the Well. Though the southern end of the Toroweap Fault passes between these two localities, the total displacement is insufficient to account for the present difference in elevation, and it appears

[1] The field work was supported by a grant from the Kemp Memorial Fund of the Columbia University Geology Department.