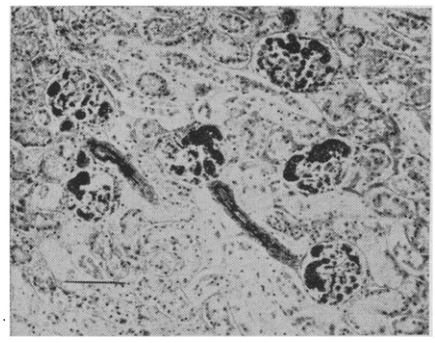

Fig. 1. Renal cortex showing marked fibrin deposition in the glomerular capillaries and early tubular necrosis (PTAH stain; scale, 100 μ).

adrenal hemorrhage, and moderate pulmonary congestion with punctate hemorrhages were less frequently encountered. The uterus invariably presented a dark appearance, owing to contained hemolyzed blood originating from placental hemorrhage. Early maceration of the fetuses was a constant feature. Portions of all tissues were fixed in 10-percent formalin, embedded in paraffin, sectioned at 7μ, and stained with hematoxylin and eosin, phosphotungstic acid hematoxylin (PTAH), and other stains. The most conspicuous feature on histological examination was the presence of a fibrillar substance blocking the renal glomerular capillaries (Fig. 1), which was identified as fibrin, on the basis of the characteristic deep-blue staining by PTAH. This finding is pathognomonic of the generalized Shwartzman reaction (*1*). Fibrin thrombi were less frequently present in the lungs, brain, spleen, liver, and adrenals. The incidence of renal glomerular fibrin thrombosis is shown in Table 1. Although the overall architecture of the intestinal mucosa was unaffected, considerable damage to the epithelium was evident in the form of disruption of cells arrested in mitosis.

Table 1. Incidence of fibrin thrombi in renal glomerular capillaries. Ratios are number of animals affected to number of animals tested.

Provocative agent	Fibrin thrombi
Pregnant hamsters	
Colchicine*	6/7
Endotoxin†	1/6
Nonpregnant hamsters (♀ and ♂)	
Colchicine‡	0/8
Endotoxin§	0/30

* Colchicine, U.S.P. (Fisher), 150 to 300 mg/kg, intraperitoneally. † Endotoxin (Bacto lipopolysaccharide, *E. coli* 055:B5, Difco), 0.1 to 0.2 mg, intraperitoneally. ‡ 300 mg/kg, intraperitoneally (twice.) § Several schedules of administration, from 0.1 mg, intraperitoneally (twice) to 0.6 mg, intraperitoneally (once).

The generalized Shwartzman phenomenon is classically produced in the nonpregnant rabbit by two suitably spaced injections of bacterial endotoxin (*1*), and it has also been described in man (*2*) and in the pregnant rat (*3*). Attempts in this laboratory to reproduce the phenomenon in the nonpregnant hamster with two spaced doses of either endotoxin or colchicine were entirely unsuccessful. Endotoxin (0.2 mg, intraperitoneally) was only feebly effective in the pregnant hamster (Table 1) although the particular batch of endotoxin used possessed good Shwartzman-provoking activity in the pregnant rabbit. Administration of colchicine (300 mg/kg of body weight, intraperitoneally) to male hamsters carrying a choriocarcinoma of human origin in the wall of the cheek pouch resulted in the development of profound lethargy which was prevented by excision of the tumor immediately prior to the injection of colchicine. However, fibrin thrombi were not found. Tumor-bearing hamsters given endotoxin (0.2 mg, intraperitoneally) also became more shocked than the appropriate controls, but again fibrin thrombi failed to appear. Neither colchicine nor endotoxin, in two spaced doses, elicited the generalized Shwartzman phenomenon in the pregnant mouse, although several inbred strains and their hybrids were tested. The simultaneous administration of complement in the form of pooled guinea-pig serum failed to influence this negative result.

While it is generally conceded that intravascular coagulation, and in particular fibrin thrombosis in the renal glomerular capillaries, may be the determining event in the pathogenesis of the generalized Shwartzman reaction, there is less agreement concerning the nature of the state of preparation. Comparison of the susceptible pregnant hamster with the apparently unresponsive nonpregnant individual may yield information on this latter point.

The unique method of provocation may result from the loss of integrity of the intestinal mucosa produced as a consequence of the colchicine-induced mitotic arrest, thus permitting endotoxin derived from the native gut flora to enter the body. Adult members of a number of species possess demonstrable immunity to the Gram-negative bacilli composing their gut flora (*4*) and a suitable state of immunologic sensitivity directed towards the provocative agent may be a prerequisite for the successful precipitation of the generalized Shwartzman phenomenon in the prepared animal (*5*). An alternative mechanism may be connected with the liberation of the etiologic agent by the damaged placenta, although the relatively weak Shwartzman-provoking activity of endotoxin would tend to discount this possibility, as endotoxin, which is known to possess a powerful abortifacient action (*6*), gave rise to considerable placental injury in the present series (*7*).

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Deoxyribonuclease Sensitivity of Ribonucleic Acid Synthesizing System from Tobacco Leaves

Abstract. *A fraction from tobacco leaf cells, containing nuclei and capable of synthesizing ribonucleic acid in the presence of the four ribonucleoside triphosphates, was inactive after incubation with deoxyribonuclease, suggesting that deoxyribonucleic acid plays an essential role in the reaction. Almost complete inhibition was obtained even with concentrations of deoxyribonuclease which removed less than half the original acid-insoluble deoxyribonucleic acid.*

In a recent report, Bandurski and Maheshwari (*1*) demonstrated the ability of a nuclear fraction from tobacco leaves to incorporate nucleoside triphosphates into ribonucleic acid (RNA). RNA-synthesizing systems have been obtained from pea embryos, animal cells, and bacteria (*2*), and they are dependent upon deoxyribonucleic acid (DNA) for activity.

In this laboratory, an extract prepared from tobacco leaves also incorporated labeled adenosine triphosphate (ATP) into RNA in the presence of Mg^{++} and the other three triphosphates. The labeled product was insoluble in cold acid and ethanol (70 percent), but it was soluble in hot NaCl solutions. It was rendered dialyzable by ribonuclease but not by deoxyribonuclease. In later studies, reported herein, incubation of nuclei-containing fractions with deoxyribonuclease inhibited their ability to incorporate ATP into RNA and suggests that DNA plays an essential role in this system as in those from animal and bacterial sources.

The enzyme fraction was obtained from leaves of young, vigorous *Nicotiana tabacum* L. (var. Havana 38) plants. Midribs were removed, and the leaves were rinsed in water, blotted, and chilled. All subsequent operations were carried out at 2°C as rapidly as possible. The leaves were ground for 2 minutes in a blender in 4 volumes of homogenizing medium (0.25*M* sucrose and 1m*M* $MgCl_2$ in 0.05*M* Tris buffer, *p*H 8.0). The homogenate was filtered through a cheesecloth-flannelette pad, and the filtrate was centrifuged at 330*g* (av.) for 4 minutes. The pellet was washed once with homogenizing medium and once with 0.25*M* sucrose in 0.05*M* Tris buffer (*p*H 8.0). It was finally suspended in a small volume of 0.05*M* Tris buffer, *p*H 8.0. Suspensions were usually dialyzed overnight against buffer at 2°C.

Microscopic examination of aliquots of these fractions stained with methyl green-pyronin revealed nuclei and chloroplasts (ratio about 1:10) and starch particles. Other aliquots for protein, DNA, and RNA analyses were washed and extracted essentially according to the method of Ts'o and Sato (*3*). Preparations from 100 g of leaves contained approximately 10^8 nuclei (hemocytometer counts), 75 mg of protein, 5 mg of DNA, and 0.1 to 0.5 mg of RNA. Protein was determined by biuret (*4*), DNA by diphenylamine (*5*), and RNA by optical density at 260 mμ.

The incorporation reaction mixture consisted of the following (in micromoles per milliliter): KCl, 60; $MgCl_2$, 5; potassium phosphate (*p*H 8), 5; mercaptoethanol, 10; Tris buffer (*p*H 8), 75; ATP-8-C^{14} (*6*), 0.5; cytidine, guanosine, and uridine triphosphates, 0.5 each; phosphoenolpyruvate, 5; pyruvate kinase (*7*), 20 μg. This mixture was incubated with 1.5 ml of the enzyme fraction in a total of 3.0 ml per tube for 30 minutes at 37°C. After incubation, the reaction was stopped with cold trichloroacetic acid, and washed and extracted by the method of Weiss (*2*). In addition, the alcohol precipitate was dissolved in 2 ml of water containing unlabeled ATP (1 mg/ml), reprecipitated with ethanol, washed twice with cold 5 percent trichloroacetic acid, and finally dissolved in 2.0 ml water. Radioactivity was determined on aliquots in a gas flow counter.

Table 1. Sensitivity to deoxyribonuclease (DNase) of RNA-synthesizing enzyme preparation from tobacco leaves. Incorporation results corrected for zero incubation time values.

Treatment	ATP-8-C^{14} incorporation (μμmole/tube)	DNA in enzyme fraction* (%)
Expt. 1, 7.6 × 10^6 *nuclei per tube*		
DNase, 50 μg/ml	1.1	1
Control	28.1	100
Expt. 2, 1.8 × 10^7 *nuclei per tube*		
DNase, 1 μg/ml	0.3	57
Control	87.2	98
Expt. 3, 1.4 × 10^7 *nuclei per tube*		
DNase, 50 μg/ml	2.1	0
DNase, 5 μg/ml	4.2	0
DNase, 0.5 μg/ml	3.2	18
DNase, 0.05 μg/ml	3.2	60
Control	93.2	90

* DNA remaining in DNase-treated and control fractions expressed as percentage of that in nonincubated aliquots.

To examine the possible role of DNA in this reaction, enzyme fractions in 0.05*M* Tris buffer (*p*H 7.0) were incubated with deoxyribonuclease (*8*) and 0.01*M* $MgCl_2$ for 1 hour at 37°C. These and fractions incubated without added enzyme were then dialyzed overnight against 0.05*M* Tris buffer (*p*H 8.0) at 2°C and used in incorporation experiments. The deoxyribonuclease preparation contained no detectable ribonuclease activity.

Incubation of the enzyme fraction with 0.05 to 50 μg of deoxyribonuclease per milliliter virtually eliminated its ability to incorporate labeled ATP into RNA (Table 1). The lower concentrations of deoxyribonuclease tested reduced less than half the native DNA in the enzyme preparations to an acid-soluble, dialyzable state. Information on the established role of DNA in the activity of RNA polymerase offers two attractive possibilities which may explain this apparent failure of the remaining DNA to support the incorporation of ATP into RNA.

Heat-denatured DNA is less efficient than native DNA in priming bacterial RNA polymerase activity, suggesting that DNA is most active in its native, double-stranded form (*9*). Thus, partial deoxyribonuclease damage to native DNA, which may be occurring at the lower enzyme concentrations, might render the product incapable of stimulating RNA synthesis and yet be limited enough to preserve a certain degree of polymerization. The possibility that some nuclear DNA is bound in a nucleoprotein complex must also be considered. Histone-bound DNA is inactive in the support of RNA polymerase activity (*10*) and may be more resistant to attack by low levels of deoxyribonuclease than unbound DNA. In this case, only the latter would be active in RNA synthesis and would be destroyed during incubation with deoxyribonuclease.

The occurrence of a contaminating ribonuclease in the deoxyribonuclease preparation seems unlikely because no nucleolytic activity was detected after prolonged incubation of the enzyme with RNA. In addition, fractions treated with deoxyribonuclease contained no less total RNA than nontreated controls.

These data demonstrate a requirement for DNA in RNA synthesis with nuclei-containing preparations from tobacco leaves, though conclusive evidence must await purification of the enzyme and an examination of the compositions of the DNA "primer" and the reaction product. It has not yet been established whether the nucleotide incorporation with this system is terminal or internal (*11*).

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