charides which, in contrast, have anti-coagulant activity, such as chondroitin sulfuric acid B(7) be liberated as well? Such reasoning led us to study the effects of heparin and polyglucose sulfate, in addition to the well-known action of dicoumarol on the coagulation mechanism. The results obtained with these materials clearly involve the blood clotting mechanism, for each produced hemorrhage in AAN-treated animals but by themselves did not. Little would be gained by discussing the mode of action of these materials. The problem is far too complex; in order to elucidate it completely each of the many blood clotting factors which are altered by these agents would have to be investigated. It seems necessary to add that in addition to coagulation factors *per se*, on the clotting mechanism, the role of heparin or dicoumarol on the integrity of the vascular walls themselves must also be kept in mind.

Another outcome of these experiments is of interest to the student of osteogenesis. For as already described, the hemorrhage dissects away the periosteal layer of osteoblasts and hence, affords a means of studying new bone formation by these cells when the effects of the lathyrogenic agent and accessory factors such as heparin or papain have worn off.

Summary. When appropriate amounts of the lathyrogenic agent, AAN, are administered together with papain or the anti-coagulants, heparin or polyglucose sulfate, widespread spontaneous subperiosteal hemorrhages are seen. These do not occur when these materials are administered by themselves. The possible underlying mechanisms of this change are discussed.

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Tobacco Mosaic Virus Reconstitution Using Inactivated Nucleic Acid.* (25163)

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The conditions required for inactivation of Tobacco Mosaic Virus ribonucleic acid (TMV-RNA) by formaldehyde, nitrous acid and other reagents have been described(1-5). It was clearly shown that in each case the rate of inactivation is considerably greater for the RNA than for the intact virus, and with several reagents first order kinetics of inactivations were observed for the RNA, while the virus appeared to become progressively more resistant. The question then arose whether inactivated RNA could combine with native TMV protein to give stable virus rods. For, such reconstituted inactive virus might be expected to represent a perfect antigen for purposes of immunization against the virus.‡ In contrast to the chemically inactivated virus, the protein of which is of

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‡ The viral protein alone, free from nucleic acid, might be assumed to represent the ideal agent for immunization. However, Aach(6) has recently shown that the dissociated TMV protein differs serologically from intact TMV, each having an antigenic component not shared by the other. Reaggregated protein rods reacted serologically like TMV, but these are not stable under physiological condi-

necessity extensively modified, the reconstituted virus would consist of native unreacted protein encasing a thoroughly modified RNA. The present experiments have shown that rods indistinguishable from TMV are obtained in good yield from propylene-oxide inactivated RNA and TMV-protein. A serological comparison of this inactive material, and its antibody, with active TMV and its antibody showed the 2 virus preparations to be indistinguishable. Other RNA derivatives were also found to reconstitute, giving inactive virus rods with similar or slightly lesser yields than are obtained in infective form from active RNA.

Methods and materials. TMV-RNA was prepared by the detergent method(7), or by means of phenol with versenate buffer (ethylenediamine tetraacetate)(8): the protein by the acetic acid method(9). The reaction with propylene oxide (10% by vol) was performed at 0°, sometimes in the presence of 10^{-4} M pH 7 versenate. After various reaction times the RNA was precipitated and reprecipitated once or twice with 2 volumes of alcohol and .05 ml 3 M sodium acetate/3 ml 67% alcoholic solution. Reconstitutions were carried out at 30° with 0.1% protein and .005% RNA in 0.1 M pH 7.3 pyrophosphate (6 hours). After one low-speed centrifugation, the reconstituted virus in an aliquot of the supernatant was sedimented at 40,000 rpm, redissolved in water, brought to 10^{-3} M with tris-buffer and again sedimented at 40,000. The redissolved pellet was analyzed by UV spectrophotometry (O.D. max 0.27 = 0.01% virus) and at times for phosphorus and arginine content. Infectivity assays were carried out with the RNA prior to and after reconstitution, as well as with the finally separated sedimentable material. The concentration of untreated RNA in test solutions required to obtain about 20 lesions/half leaf is usually 0.1 to 0.5 γ/ml. Thus tests for more than 99.9% inactivation (requiring over 0.5 mg RNA/ml) become progressively more costly and impracticable. However, reconstitution increases sensitivity of test so that 10^{-3} γ/ml RNA are readily detected, and absence of lesions after application of a solution containing 1% reconstituted virus (.5 mg RNA/ml) would signify inactivation by a factor of 10^{-6} to 10^{-7}. For preparation of the antiserum (anti-P.O.-TMV-serum), rabbits were injected with increasing amounts of reconstituted TMV (0.2-1.2 mg/day, prepared from propylene oxide-inactivated RNA) 11 times over a 3 week period, and bled 10 days after last injection. For production of anti-TMV-serum, daily doses of 0.5-2 mg were generally used. The application of light scattering to quantitative evaluation of the virus-antibody reaction will be described elsewhere(10).

Results. TMV-RNA is inactivated by 10% propylene oxide at 0° at the rate of approximately one log/hour (Table I). The yield of 99% inactivated sedimentable material obtainable from this RNA by reconstitution with protein is similar to that obtained from the untreated RNA, and decreases only gradually upon more extensive modification of the RNA. Electromicrographs§ of such material showed only rods of typical diameter; about 30-50% of the mass was usually in the form of rods of 260-300 mμ length.

The interaction of TMV and inactive, reconstituted TMV with their antisera was studied by light scattering technic. The virus preparations were incubated with homologous and heterologous sera in 0.1 M pH 7 potassium phosphate buffer. Concentrations of the reactants were: 38 μg/ml of TMV; 34 μg/ml of inactive reconstituted TMV and 0.1 to 10 μl/ml of antiserum. After incubation at 30° for 3 hours the samples were transferred to the light scattering cell and the Rayleigh ratios at 45°, 90° and 135° (R_{45} and R_{135}) determined. The point of equivalence was obtained from a plot of the dissymmetry ratio, z (R_{45}/R_{135}), against concentration of antiserum. The dissymmetry reaches a maximum value at point of equivalence. Equivalence values obtained were: 1 ml anti-TMV-serum

tions and can, therefore, not be used as antigen. Thus, for purposes of immunization against TMV the RNA is necessary, though only as a stabilizing agent of the rod structure.

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TABLE I. Effect of Modification of TMV-RNA on Its Infectivity and Ability to Reconstitute.*

Reagent (conc.)	Reaction conditions	Infectivity, %†	Ability to reconstitute,* %†
Propylene oxide (10%)	0°, pH 7 versenate (.005M) 1 hr	22	94
	Idem 2.5 "	.9	68
	" 24 "	.002	37
	0°, (no buffer, pH 7-8) 1 "	20	71
	Idem 2.5 "	1.3	65
β-Propiolactone (.2%)	0°, 2 h, pH 7 phosphate (.025M)	.2	80
(.5%)	*Idem*	.02	58
Nitrous acid (M $NaNO_2$)	25°, .75 h, pH 4.7 acetate (.25M)	.16	85
	" 3 h " "	<.01	83
U. V. irradiation	0°, .25 h	.7	86
	" 1 h	.015	86
Ribonuclease (10^{-5})‡	25°, .5 h, pH 7 phosphate (10^{-3}M)	<.1	20
(5×10^{-6})	" " "	0–5	0–20
(10^{-6})	" " "	45–57	46–76

* Ability to reconstitute to form virus-like rods with TMV protein in 0.1 M pyrophosphate (Prot/RNA = 20), as measured by ultracentrifuge sedimentation (see *Methods*).

† % of corresponding untreated control.

‡ Enzyme/substrate wt ratio (crystalline pancreatic ribonuclease was used).

= 101 mg inactive reconstituted TMV = 115 mg TMV. 1 ml anti-P.O. – TMV serum = 6.4 mg inactive reconstituted TMV = 7.4 mg TMV. The difference between these values is not significant and the 2 virus preparations were indistinguishable by this technic. The lower titer of the anti-P.O.-TMV-serum is probably due to the lesser concentrations of reconstituted virus used in immunization of the rabbits.

These data seem to show clearly that an anti-TMV serum of high serological activity can be produced with a virus of very low infectivity, obtained by reconstituting propylene-oxide treated RNA with native protein. Inactivation of intact TMV under the same conditions proceeds so slowly that no preparation of similar low infectivity (decrease by over 5 logs) was obtained, but it may be surmised that the extensive alkylation of the protein occurring in the course of the reaction would have affected its serological properties both qualitatively and quantitatively.

RNA inactivated by certain other means (*e.g.* UV light, formaldehyde, nitrous acid, β-propiolactone) also interacted with the native virus protein to give appreciable amounts of sedimentable material (Table I) which can safely be assumed to represent high-specificity viral antigen. In contrast, reactions which decreased the average nucleotide chain length of the RNA, in particular traces of ribonuclease, quickly abolished its ability to form sedimentable material.

It has previously been reported that nucleic acid of a greatly differing strain (HR) gave poor yields upon reconstitution with common TMV-protein, while the opposite combination (HR protein, TMV-RNA) was less disadvantageous(10). We are thus faced with an interesting structural problem: what is believed to be only a different sequential arrangement of the same bases seems to cause enough steric hindrance to interfere with satisfactory RNA-protein interaction while substitution or chemical alteration of bases does not necessarily affect their ability to enter into rod formation. However, it is possible that the extent of chemical modification required for inactivation may be quite low, and therefore localized, while sequential differences of strains may be recurring throughout the chain. Unfortunately no good analytical data for the average extent of interaction of the reagents described are yet available. In a previous study with C^{14} labelled iodoacetate which reacts only slowly with RNA, about 7-10 residues were bound per mole RNA (M.W. 2 x 10^6, 7000 nucleotides) when over 90% of the infectivity was abolished (36°, 16 hours, pH 8)(11). From the kinetics of inactivation with nitrous acid

Schuster and Schramm(3) concluded that the deamination of 2 groups was, in average, lethal. It thus does seem probable that all RNA inactivating reactions need involve but a small fraction of the potentially reactive sites. The ability to coaggregate with native protein may not be noticeably affected by this extent of modification of the RNA structure.

It seems possible that these observations may have future practical implications. When the nucleic acid free proteins of pathogenic viruses will have been isolated in native form, these may prove more suitable for immunization than the "killed" viruses. If, however, they should prove less than perfect, because they lack the typical virus achitecture‡, then reconstitution of such a native protein with the isolated and thoroughly inactivated preparation of the corresponding RNA might supply the most effective means of safe immunization. Unfortunately, reconstitution has yet been possible only with TMV and its strains.

Summary. TMV-RNA inactivated by various means, in particular by reaction with propylene oxide, retains its ability to form virus-like rods with native TMV-protein. Such reconstituted inactive virus is serologically indistinguishable from the intact virus. The structural and potential practical implications of this observation are discussed.

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Correlation between Oxygen Consumption and Erythropoiesis in Hypophysectomized Rats Treated with Various Doses of Thyroxin.* (25164)

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Anemia which develops in hypophysectomized animals is accompanied by a decrease in oxygen consumption(1-4). Therapies which have eliminated this anemia, such as a combination of thyroxin, cortisone and growth hormone(3,4), have elevated oxygen consumption. It has been proposed(1,2) that anemia in the hypophysectomized rat is an expression of a decreased need for oxygen of the animal, and that the above therapy proved beneficial because of increased oxygen need resulting from such treatment. This was tested by altering oxygen consumption with various doses of thyroxin given in conjunction with cortisone and growth hormone(4). Oxygen consumption and erythropoiesis varied directly with the dose of thyroxin given. Several investigators studied the effect of thyroxin on hypophysectomized rats(5-8). Partial alleviation of anemia, bone marrow repair and reticulocytosis have all been reported but no attempts were made to relate the effect of various doses of thyroxin to changes in oxygen consumption and alterations in erythropoiesis. If the oxygen need theory is correct, thyroxin

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