ing. The incidence of these ulcers was greatest at 24 hours, which coincides with the most pronounced fall in fibrinogen values. Cold, therefore, could have a specific action of depressing the fibrinogen production. It is equally possible that the plasma fibrinogen fall represents only another particular instance of the generalized protein catabolism during exposure to cold. Thus, an increased catabolism of fibrinogen, if unaccompanied by a corresponding increase in its production, appears to result in a fall of its concentration in the plasma.

Summary. In rats fasted for 24 hours before bleeding, exposure to cold causes a fall in the plasma fibrinogen concentration. The fall is maximal at the end of the first 24 hours and is followed by a slight rise to a subnormal level. This low level is maintained for as long as 21 days after beginning of cold treatment.

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17089 P. Inhibition of Multiplication of Influenza Virus by Extracts of Tea.*

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It was reported[1] previously that tannic acid inhibits the multiplication of influenza A virus in embryonated eggs. The tannic acid used was tannic acid, USP, a commercial sample, presumably extracted from nutgalls. During the course of studies designed to test the inhibitory effect of tannic acid obtained from other sources, attempts were made to extract a suitable preparation of tannic acid from tea. Preparations having certain of the characteristics of tannic acid and showing inhibitory activity have been obtained; in addition, other preparations differing, in some respects, from tannic acid and showing somewhat greater inhibitory activity than the "tannic acid" fractions also have been extracted from tea.

Materials and methods. The tea employed was a commercial sample of black tea. The PR8 strain of influenza A was used exclusively. Materials to be tested were dissolved in water, autoclaved, and neutralized with sodium hydroxide. The specified amount, in a volume of 0.5 cc, was injected into the allantoic sac of 10-day embryonated eggs and after an interval of one-half hour approximately 100 ID_{50} of virus were inoculated by the same route. Inhibition of multiplication was considered to have occurred if, after 48 hours incubation, allantoic fluid from eggs so-treated, did not agglutinate chicken erythrocytes.

Experimental. A desiccated hot water extract of tea was prepared and fractionated by the following methods: (1) by solubility or insolubility in absolute ethyl alcohol, (2) by fractional precipitation from aqueous solution with various concentrations of ethyl alcohol, (3) by precipitation from aqueous solution on addition of sufficient dilute HCl to reduce the pH to 2-3. Fractions thus obtained were desiccated and injected into groups of embryonated eggs, in amounts increasing by two-fold increments, one-half hour before inoculation of virus. The amount of each fraction per egg necessary to inhibit virus multiplication in 50 per cent of the eggs was then determined. In some instances, toxicities in terms of LD_{50} also were determined.

Inspection of Table I reveals that all fractions showed some inhibitory activity. Moreover, activity may be concentrated by removal of alcohol soluble materials, by precipitation at 88 per cent alcohol, and by precipitation at pH 2-3. Tannic acid USP is approximately twice as active but four times more toxic than

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1 Green, R. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 483.

TABLE I.

Fraction No.	Description of fraction	Inhibitory activity: Total No. of eggs	Inhibitory activity: Amt necessary for 50% inhibition, mg per egg	Toxicity: Total No. of eggs	Toxicity: Amt necessary for 50% mortality, mg per egg
1	Hot water extract	71	0.91		
2	Alcohol soluble	43	2.00	25	7.0
3	Alcohol insoluble	27	0.66		
4	Ppt. at 64% alcohol	44	1.59		
5	Ppt. at 65 to 80% alcohol	66	0.84		
6	Ppt. at 81 to 88% alcohol	79	0.41	32	6.6
7	Soluble at 88% alcohol	36	0.92		
8	Ppt. at pH 2-3	29	0.46		
9	Soluble at pH 2-3	21	1.20		
10	Tannic acid, USP	112	0.22	32	1.6

the most active tea fraction, that precipitated at 81 to 88% alcohol (Fraction 6).

Because of its greater activity fraction 6 was chosen for more detailed study. Repeated precipitation with 88% alcohol at pH 5-6 resulted in no significant change in activity. *In vitro* tests, carried out as previously described,[1] show that as little as 25 μg per cc will inactivate a significant amount of virus. Two mg of fraction 6 per egg inhibit multiplication when given as long as 2 hours before or 2 hours after inoculation of virus. Injection of 10 mg into the yolk sac 2 hours before inoculation of virus into the allantoic sac does not inhibit multiplication.

Discussion. Obviously, attempts at purification have been crude, and probably the fractions prepared are mixtures of uncertain composition. Some fractions should contain tannic acid as an appreciable amount is present in tea.[2] Furthermore, as tannic acid is said to be soluble in alcohol, one would expect any present to be concentrated in the alcohol soluble fractions (No. 2 and 7). If inhibitory activity were due to tannic acid the alcohol insoluble fraction (No. 3) and that precipitated at a concentration of 81 to 88% alcohol (No. 6) should show relatively little activity. However, the reverse is true, these fractions being among the most active ones obtained. These differences in solubility may be more apparent than real, depending upon other variables such as pH, which have not been investigated fully. Common tests for tannic acid, such as those employing ferric salts and soluble protein, which are notoriously non-specific, have failed to differentiate clearly the various fractions from each other and from tannic acid. The difficulty of deciding whether the active principle is or is not tannic acid or one of the tannins is further emphasized by the fact that the different natural tannins exhibit differences in their chemical reactions and behavior with solvents.[2] Furthermore, even the "purified" or "crystalline" preparations of tannic acid are mixtures, as it is well recognized that they are contaminated with various impurities.[3] Although the effect of fraction 6 on influenza virus is similar to that of tannic acid, the most significant difference may be a biological one, namely, that the former, although only one-half as active as tannic acid, is some four times less toxic.

Summary. Extracts of tea have yielded an unidentified fraction, similar to but less toxic than tannic acid USP, which inhibits the multiplication of influenza virus in embryonated eggs.

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[2] Nierenstein, M., Tannins, in Allen's Commercial Organic Analysis, Vol. V, 5th edition, P. Blakiston's Son and Co., Philadelphia, 1927.

[3] Nierenstein, M., The Natural Organic Tannins, J. and A. Churchill, Ltd., London, 1934.

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