17088. Influence of Cold on Blood Fibrinogen Concentration.*

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Changes of several chemical constituents of the blood are known to follow the same pattern under diverse conditions of non-specific stress. These biochemical changes have therefore been considered to be manifestations of the general adaptation syndrome. A survey of the literature suggests that perhaps variations in blood fibrinogen belong to this same group. Thus an increase in plasma fibrinogen has been reported in many non-specific stress conditions, such as infectious diseases[2] and a wide variety of chemical and physical injuries.[3,4,5]

On the other hand, a decrease in plasma fibrinogen appears to be almost always associated with specific disturbances directly related to its production, such as dietary deficiencies[2] or extensive liver damage.[5]

We therefore decided to study the blood fibrinogen changes occurring in rats exposed to cold, during the stages of alarm and resistance of the "general adaptation syndrome."

Material and Methods. Eight groups of 8 female piebald rats, weighing 130-165 g (average 145 g) were used in this experiment. All animals were put into the cold room (2-5°C) in individual cages, except 2 groups kept at room temperature as controls. All groups were maintained throughout on Purina Fox Chow, with tap water *ad libitum*. The 6 groups of rats were removed from the cold room, bled and killed, after 10, 24, 48 hours and 5, 10, 21 days respectively. During the last 24 hours of exposure all animals were fasted, but water was allowed. Blood samples were taken by carotid puncture under ether anesthesia. Blood coagulation was prevented by the use of silicone-coated syringes and needles, the samples being added to tubes containing 3 mg of potassium oxalate per ml of blood.

At the end of the experiment, one group of control rats was bled and killed without fasting, while the other group was fasted for 24 hours before bleeding.

At autopsy all the animals were carefully inspected for any detectable infection. The adrenals and thymus were removed, fixed in formalin and weighed.

The plasma fibrinogen was determined by a modification of the method of Campbell and Hanna[6] with direct nesslerization. In order to avoid the digestion in centrifuge tubes which frequently causes loss of material by bumping, we modified it as follows: the precipitated fibrinogen was quantitatively transferred with distilled water to a Folin 50 ml graduated digestion tube. The tube was then placed in an oven at 90-100°C overnight in order to evaporate the water. The following day the dry residue was digested with 1 ml of the undiluted digestion mixture. Decoloration frequently occurs following nesslerization due to the presence of an inert precipitate formed during digestion. This was prevented by diluting the digest exactly to 50 ml with distilled water, centrifuging the solution and pipetting 25 ml of the clear supernatant. The nesslerization was then performed in a 50 ml cylinder. To avoid the turbidity which occasionally occurs during the nesslerization, the diluted digest was made up to 35 ml with distilled water and the cylinder immersed in a cold bath for several minutes. Then 15 ml of the Nessler's reagent (Folin's formula) was quickly added, mixed immediately by inversion and the cylinder maintained in the water bath until read in the colorimeter. All determinations were made in duplicate.

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2 Ham, T., and Curtis, F. C., *Medicina*, 1938, **17**, 413.

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4 Chanutin, A., and Ludewig, S., *J. Biol. Chem.*, 1947, **167**, 313.

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TABLE I.
Blood Fibrinogen and Adrenal and Thymus Weights During Adaptation to Cold.

No. of animals	Body wt		Time of exposure	Adrenal wt, mg	Thymus wt, mg	Plasma fibrinogen, mg/100 ml
	Initial	Final				
8*	145 ± 10.5†	—	0	27.7 ± 1.20‡	271 ± 18.0‡	252 ± 12.6‡
7	147 ± 8.9	138 ± 8.2	0	34.3 ± 1.94	163 ± 9.1	257 ± 9.3
8	146 ± 9.7	127 ± 10.3	10 hr	36.1 ± 1.50	156 ± 16.8	242 ± 15.3
7	148 ± 11.4	129 ± 9.5	1 day	35.4 ± 1.18	117 ± 9.3	191 ± 5.9
8	149 ± 12.7	127 ± 13.2	2 "	39.4 ± 1.26	108 ± 3.9	206 ± 11.1
8	148 ± 11.7	122 ± 9.0	5 "	37.7 ± 1.86	67 ± 5.2	208 ± 7.6
8	149 ± 10.0	128 ± 12.0	10 "	42.3 ± 1.95	91 ± 7.9	207 ± 6.5
8	148 ± 11.5	138 ± 16.3	21 "	43.4 ± 3.87	90 ± 14.2	208 ± 13.4

* Not fasted before fibrinogen determination.
† Standard deviations.
‡ Standard errors.

Results. Table I summarizes our results. The changes in blood fibrinogen and adrenal and thymus weights during the experimental period are also shown in Fig. 1. The values at zero time are those of the 24 hour fasted controls. The blood plasma fibrinogen decreased sharply from the control value of 257 mg to 191 mg per 100 ml in the first 24 hours. At the end of 48 hours the fibrinogen increased to 206 mg, remaining at this level until the end of the experimental period of 21 days. As can be seen from the table, fasting alone did not influence the blood fibrinogen concentration.

The weight of the adrenals increased rapidly in the first 48 hours and at a slower rate for the remainder of the cold exposure period. The thymus weights show a decrease, which was most marked at the end of the fifth day of exposure to cold, continuing approximately at the same level till the end of the experiment. The weights of these organs were used as an index showing the efficacy of the alarming stimulus. The changes observed are those constantly present in the stages of alarm and resistance of the "general adaptation syndrome".

Discussion. It seems apparent that the plasma fibrinogen does not respond in a constant manner to different types of stress as other constituents of body fluids do. In all the previously mentioned instances of blood fibrinogen increase the common feature was the tissue damage, which is known to be a stimulus for fibrinogen production. In this connection, it is noteworthy that the presence of a fibrinogen-production-stimulating substance in a turpentine-induced abscess in dogs has also been reported.[7]

In the present experiment tissue damage was not excluded. Apart from the intense catabolism indicated by the marked loss of weight, most of the animals developed severe gastric ulcers when exposed to cold and fast-

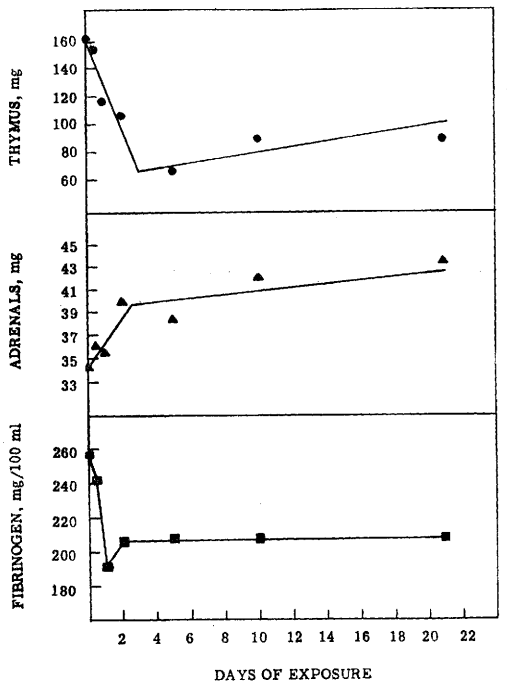

FIG. 1.

Blood fibrinogen, adrenal and thymus weights during adaptation to cold.

[7] Homburger, F., *J. Clin. Invest.*, 1945, **24**, 43.

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.