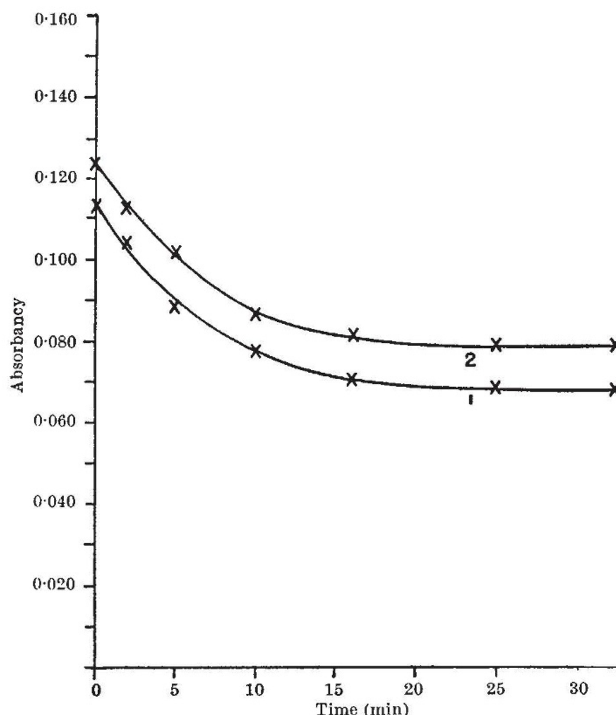

Fig. 1. Absorbancy as a function of time for the sulphydryl group remaining in 0·1 ml. of human blood on incubation of 1 ml. of blood at 37° C with 1 mg of epinephrine

EDTA as an anticoagulant yielded the same results as heparin. When 20 μg of glutathione in either 1 ml. of trichloroacetic acid or 1 ml. of water was added to Ellman's reagent, an average net absorbancy reading of 99, with a standard deviation of 7, was obtained in six determinations, the absorbancy of the PNPD blank without glutathione being six. During 30 min of incubation at 37° C in the absence of epinephrine, the non-protein sulphydryl group of blood did not change. Plasma did not effect the disappearance of the sulphydryl group significantly. Indeed, the net absorbancy of the —SH group in 1 ml. of the trichloroacetic acid filtrate formed from 1 ml. of plasma was only six. Furthermore, in contrast to the behaviour in blood, half of 200 μg of glutathione added to 1 ml. of plasma disappeared in 20 min, and 1 mg of epinephrine inhibited the decrease appreciably.

In investigations concerning the mechanism of the glutathione reaction, the epinephrine was added not to blood but rather to 1 ml. of blood trichloroacetic acid filtrate the *p*H of which had been adjusted to 7·5 by addition of 0·3 ml. of a 1·1 M EDTA solution. The results were essentially the same as in Fig. 1. Epinephrine did not cause disappearance of the —SH group below *p*H 5·2 within 30 min, while at *p*H 5·8 the disappearance was the same as at *p*H 7·5. The pink colour and the increased absorbancy at 480 mμ produced by as little as 4 μg of adrenochrome failed to appear as the —SH group decreased. Ascorbic acid had a marked effect on the disappearance of the —SH group in blood and filtrate. Addition of 3 mg of ascorbic acid to 1 ml. of blood immediately before 1 mg of epinephrine prevented disappearance of the —SH group for 30 min. One mg of ascorbic acid prevented the disappearance for 15 min; but nearly the full decrease in the —SH group occurred after 15–30 min. Similar results were obtained in the filtrate.

The non-protein sulphydryl group of the red cell is largely glutathione[7]. The recovery of glutathione added to blood was nearly 100 per cent in the Ellman technique, while added cysteine and ergothionine were not at all recovered[7]. Since the sulphydryl group of blood is present almost solely within the red cell, the progressive decrease in its concentration during incubation with epinephrine must have resulted from intracellular reactions. Only a small portion of the added epinephrine actually affected the sulphydryl group. Use of relatively large amounts of epinephrine was necessary to permit reasonably large changes in the absorbancy produced from the sulphydryl group. Sensitive methods, such as those dependent on radioactivity, should enable the results of the present investigation to be extended without difficulty to physiologically significant levels of epinephrine.

The inhibitory effect of ascorbic acid suggested that the disappearance of the —SH group involved oxidation of the epinephrine. Decrease in the —SH group on incubation of the protein-free filtrate with epinephrine indicated that the oxidizing agent was not a protein. It required a *p*H higher than 5·8 for full effectiveness. Its action was rapid but inadequately strong to produce any adrenochrome from the large excess of epinephrine, in contrast to manganese dioxide. The disappearance of erythrocyte glutathione in the presence of epinephrine in the present investigation was compatible with similar behaviour in the intact living organism. These observations might equally be applicable to the cells of such organs as the liver and kidney, in agreement with the finding that repeated injection of epinephrine into rats over 4·5 h led to a decrease in the glutathione —SH group within these organs[8]. The mechanism of the sulphydryl disappearance, including its relationship to the formation of presently known metabolic products of the catecholamines, such as metanephrine and normetanephrine[5], remained to be determined.

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Panagglutination of Erythrocytes coupled with Diazotized *p*-Nitroaniline

MODIFICATION of erythrocytes *in vitro* by various enzymatic or chemical treatments renders them agglutinable by normal sera from mature donors. This agglutination reaction is called 'panagglutination'[1,2]. In the course of our investigation on the specific reaction of azohapten-erythrocytes with antisera against azohaptens[3], we observed that after coupling erythrocytes with diazotized *p*-nitroaniline (PNA), they were agglutinated by normal rabbit serum absorbed with untreated erythrocytes[3]. This communication deals with the finding of a similar phenomenon with human serum.

PNA-erythrocytes were prepared by adding 0·5 ml. of 0·005 M *p*-nitrobenzenediazonium chloride to a suspension of 0·05 ml. three-times washed packed human erythrocytes in 4·5 ml. phosphate buffer (0·15 M, *p*H 7·3) with immediate mixing. After standing for 15 min at room temperature, the coupled cells were washed twice with 10 ml. physiological saline, containing 1 per cent decomplemented rabbit serum absorbed with untreated human red cells, or 0·06 per cent human serum albumin, and then resuspended in 2·5 ml. of the same solution. To obtain consistent results, the erythrocytes used for coupling should be freshly drawn, and tested within 0·5–1 h after coupling. Normal and pathological human sera and

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plasma were obtained by venipuncture and used immediately, or stored at −25° C. The sera used were either blood group compatible, or absorbed with a sample of the same cells which were afterwards coupled with PNA. Titration was performed on a glass plate as already described[3]. Controls with untreated erythrocytes, as well as controls of the coupled cells with saline only, were always included. Serum titres are expressed as the reciprocal of the highest dilution giving agglutination.

The results obtained with 235 human sera are summarized in Table 1. From the pattern of agglutination observed, it appears that the titre is raised in patients with hepatitis and anæmia as compared with sera from healthy donors. The titre is also raised in pregnant women. It is lowered, on the other hand, in patients with leukæmia. However, the number of sera is too limited to allow any definite conclusions. Inhibition tests with PNA-tyrosin and absorptions with insoluble PNA-γ-globulin as described in ref. 3 were performed and induced no appreciable reduction of the agglutination titres. This indicates that an agglutinin with a specificity directed against the hapten PNA is not involved in this reaction. Highly active $H(O)$-substance also failed to show an inhibitory effect. The agglutinating factor could be absorbed from the sera with PNA-erythrocytes, but not with untreated erythrocytes. The activity of this factor was not changed by heating the serum to 56° for 30 min; it was significantly reduced after 30 min heating at 62°, and completely destroyed after 30 min at 65°. All the agglutinating activity could be precipitated by 45 per cent saturation of the serum with ammonium sulphate[4]. The precipitate was dissolved and made up to the original volume in physiological saline. Tested against PNA-erythrocytes, it gave the same titre as the serum from which it was prepared. By paper electrophoresis, it was shown to contain 70–80 per cent β- and γ-globulins, small amounts of α_2- and traces of α_1-globulin.

Table 1

Serum donors	No. of sera tested	No. of sera having a titre of: <1	1	2	4	8	16	32	64	128	>128
Human (healthy)	50	1	4	10	14	11	7	2	1	0	0
Human (pregnant)	78	0	2	9	21	24	13	6	2	1	0
Human (anæmic*)	33	1	5	2	7	11	4	1	1	1	0
Human (leukæmic†)	16	4	2	4	2	0	1	1	2	0	0
Human (hepatitic)	21	0	0	3	1	5	5	1	4	1	1
Human (various other diseases‡)	37	0	2	7	13	6	6	3	0	0	0
Guinea-pig (healthy)	10	0	0	1	6	2	1	0	0	0	0
Rabbit (healthy)	10	0	1	5	2	2	0	0	0	0	0

* Hæmolytic, pernicious or iron deficiency anæmia.
† Lymphocytic and myelocytic leukæmia, lymphogranulomatosis.
‡ Including polyarthritis, rheumatic fever, nephritis, nephrosis, liver cirrhosis and jaundice.

Rabbit and guinea-pig sera and erythrocytes, obtained by carotic bleeding, were also tested. The animal sera were always carefully absorbed with an untreated sample of the cells which were afterwards used for coupling with PNA. After absorption, these sera did not agglutinate the untreated cell control, but did agglutinate human PNA-erythrocytes. The titres were somewhat lower than for human sera (cf. Table 1).

All tested human red blood cells could be rendered panagglutinable without respect to their blood group. When erythrocytes from various donors were tested against the same serum, the titres were identical. The same titre was also obtained when the serum donor's own red cells were used. The PNA-treated erythrocytes are brown in colour and show a high tendency to hæmolyse. When other diazotized amines were used for coupling, as, for example, p-sulphanilic acid or p-aminobenzoic acid, panagglutination titres were significantly lower or absent. However, in these cases, the treated cells did not show such an increased tendency to hæmolysis. Rabbit and guinea-pig PNA-erythrocytes usually gave negative results when tested against human, rabbit and guinea-pig sera; only in a few cases were low titres obtained. All tested human and animal PNA-erythrocytes gave a high titre with a specific rabbit anti-PNA serum.

Since no serological relation to the coupling agent, to $H(O)$-substance, or to blood group agglutinogens could be demonstrated, the agglutination of PNA-erythrocytes appears to be a typical panagglutination. As such, it would be comparable with the agglutination of erythrocytes treated with the enzymes neuraminidase[5] or trypsin[6], which is thought to be due to an uncovering of pre-existing receptors on the red cell membrane, or with the agglutination of erythrocytes treated with the oxidizing agent periodate[7].

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Appearance of Slow α_2-Globulin during the Inflammatory Response of the Rat

Slow α_2-globulin is a protein of large molecular weight which becomes demonstrable by vertical starch-gel electrophoresis in the serum of the rat under a variety of conditions, mostly related to growth. It cannot be demonstrated by this means in healthy, non-pregnant adult animals but has been demonstrated in connexion with normal growth[1–3], neoplastic growth[1,2,4,5], regenerative or compensatory growth[6,7], pregnancy[1,2], tight abdominal bandaging[4], Caesarean section with retention of a single placenta[1], implantation of homologous minced kidney tissue[7], unilateral kidney ligation[7] and in certain states resembling mild infectious diseases[1,6]. The appearance of slow α_2-globulin in the last few of these conditions, together with the observation that it becomes sporadically demonstrable after limb amputation or sham surgery[7], especially in the presence of obvious wound infection, led to the hypothesis that, in addition to growth, the presence of an infection and/or an inflammatory condition can elicit the appearance of slow α_2-globulin in the serum of the rat.

In order to test this hypothesis, rats were subjected to various treatments designed to produce a localized or generalized inflammatory state. Sera were obtained as previously described[2] from Sprague–Dawley rats, of either sex, raised in our laboratory or purchased from the Holtzman Co., Madison, Wisconsin. Little difference in the responsiveness of the two sexes was noted. Vertical starch-gel electrophoresis was performed by the method of Smithies[8], with minor modifications[2]. Lipopolysaccharides (endotoxins) of *Salmonella abortus equi*, *S. typhosa* and *Escherichia coli*, produced by the Westphal method[9] and obtained from Difco Laboratories, Detroit, Michigan, were administered intraperitoneally in the doses indicated in Table 1 as a 1 per cent (w/v) suspension in sterile 0·9 per cent saline. Complete and incomplete Freund's adjuvants (Difco Laboratories) were administered intraperitoneally without dilution in the doses shown in Table 1. For the evocation of a localized inflammation, croton oil (Frank W. Kerr Chemical Co., Detroit, Michigan) was administered intradermally into one or two abdominal sites as a 75 per cent (v/v) solution in peanut oil[10]. Controls

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