

citrate' was prepared by irradiating 0.015M sodium citrate + 0.15M sodium chloride (32 kilorads) under oxygen bubbling and waiting 1 h. This solution was then placed in the thermostatted cell compartment of a Beckman DU spectrophotometer, and slowly heated. The absorbance at 260 nm increased very slightly as it was heated to 55°C, apparently due to accelerated residual peroxide formation, since a portion kept as a control also increased slightly in absorption within two days, and then rapidly declined as the temperature reached 60–70°C (Figure 3). This would be the expected range of decomposition of a per-acid, as observed with peracetic acid and persuccinic acid. The unusual hyperchromic effect of irradiated DNA solution shown in Figure 1 is therefore

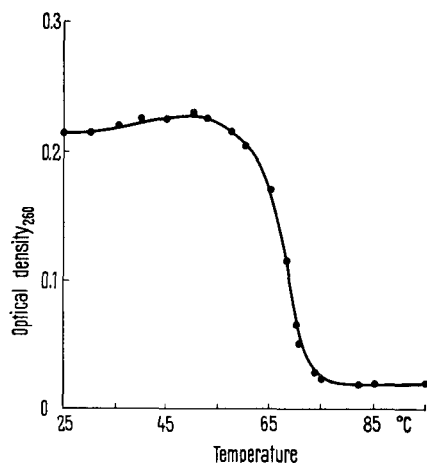


Fig. 3. Thermal decomposition curve of 'sodium peroxy-citrate' in saline citrate buffer.

due to the formation and decomposition of the product formed in the irradiation of sodium citrate.

The radiation chemistry of solutions of organic acids has been studied by others, and decomposition into hydrogen and carbon dioxide is generally observed. The yield for formic acid decomposition for example is $G = 3.2^5$, while acetic acid and glycolic acid give H_2 and dimeric products. Ascorbic acid in solution is oxidized by gamma radiation to dehydroascorbic acid with oxygen saturation enhancing the rate of destruction, but a post-irradiation reaction is not observed⁶. The reactive intermediate is presumably not a free radical but an oxygen containing species which undergoes a rearrangement to the peroxide.

In a separate experiment in which H_2O_2 was added to a 0.015M solution of sodium citrate, no increase in UV-absorption with time was observed. The results presented here indicate that a product of radiolysis of sodium citrate, a commonly used buffer material, is sodium 'peroxy citrate', formed during a post-effect. Oxygen must be present during irradiation for this product to form.

Résumé. Les résultats présentés ici suggèrent la formation d'un produit du type peroxyde après l'irradiation, en présence d'oxygène, d'une solution aqueuse de citrate de sodium tamponnée au pH 7.

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Biophysics Division, Sloan-Kettering Institute for Cancer Research, New York (N.Y., USA), August 13, 1965.

⁵ A. O. ALLEN, *The Radiation Chemistry of Water and Aqueous Solutions* (Nostrand Co., New Jersey 1961).

⁶ B. S. RAO, *Radiat. Res.* 5, 683 (1962).

Appearance of Pre- α_2 -Globulins Soon After the Very First Dose of Diphtheria Toxoid in Horse

The changes in the serum protein pattern during one course of immunization of horse with diphtheria toxoid was measured by us. The electrophoretic separation of serum proteins of horse before commencement of immunization and four days after the first dose of 5 ml of diphtheria toxoid was carried out on agar gel at pH 8.6 according to the method of GIRI¹ as modified by ACHARYA et al.². The lipoproteins were analysed according to the method of DAS and GIRI³. A component with electrophoretic mobility between α_1 - and α_2 -globulin appeared soon after the first dose of diphtheria toxoid (Figure 1). We preferred to call this component pre- α_2 -globulin. In addition to this, there occurred a slight decrease in albumin also soon after the first dose. There also appeared a new lipoprotein component with the electrophoretic mobility between α - and β -lipoprotein as seen in Figure 2. We preferred to call this component pre- β -lipoprotein. Since no detectable antibody activity was found at this stage in the α -globulin region, the increase in the pre- α_2 -

globulin soon after the first dose of diphtheria toxoid cannot be attributed to antibody formation.

Repeated injections at four day intervals for about two months showed no significant increase or decrease of pre- α_2 -globulin, in spite of the marked increase in total proteins from 6.8 to 9.6 g per 100 ml, which was found to be mainly due to increased β - and γ -globulins. But a considerable amount of antibody activity (nearly 7–8% of total activity) was also found to be associated in α -globulin region after the hyperimmunization of horse, which agrees well with the previous observation made by RAYNAUD⁴, and also by RAO⁵. The appearance of pre- β -

¹ K. V. GIRI, *J. Ind. Inst. Sci.* 38 (A), 190 (1956).

² U. S. V. ACHARYA, M. SWAMINATHAN, A. SREENIVASAN, and V. SUBRAHMANYAN, *Ind. J. med. Res.* 52, 224 (1964).

³ B. R. DAS and K. V. GIRI, *J. Ind. Inst. Sci.* 41 (A), 74 (1959).

⁴ M. RAYNAUD, in *Mechanism of Hypersensitivity*, Henry Ford Symposium (Little Brown & Co., Boston, Massachusetts 1958).

⁵ S. S. RAO, in *Advances in Biochemistry*, Proceedings of the Summer School in Biochemistry, Srinagar (1962).

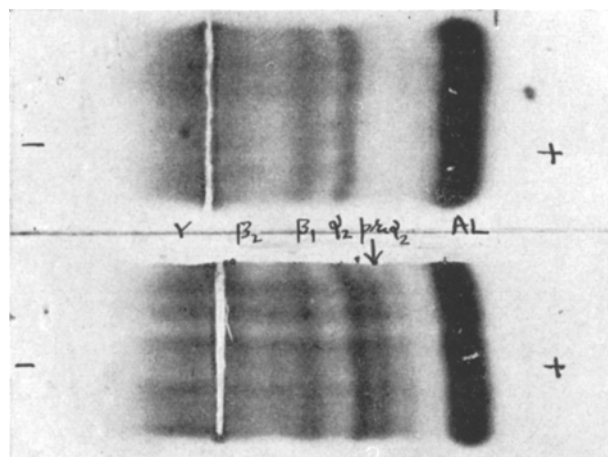


Fig. 1. Electrophoresis of horse serum proteins on agar gel at pH 8.6 before commencement of immunization (above) and after the very first dose of diphtheria toxoid (below).

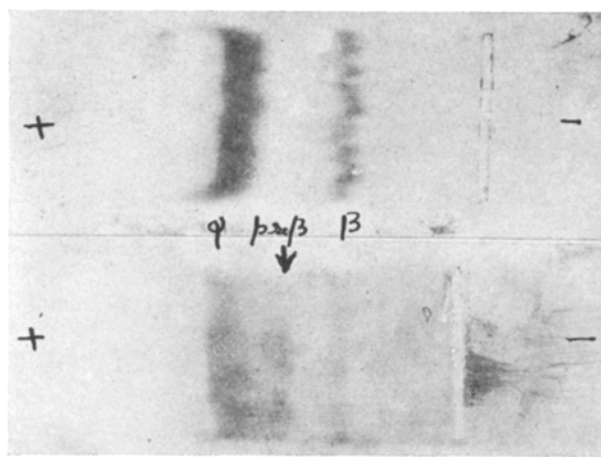


Fig. 2. Electrophoresis of horse serum lipoproteins on agar gel at pH 8.6 before commencement of immunization (above) and after the very first dose of diphtheria toxoid (below).

lipoprotein is found to be only a temporary change and the component disappeared as the hyperimmunization proceeded, whereas the pre- α_2 -globulin, which appeared soon after the first dose, remained as a permanent feature throughout the hyperimmunization.

The significance of this pre- α_2 -globulin and its function, if any, is not known. WAJCHENBERG⁶ found that the sera of patients with diffuse massive necrosis were characterized by the appearance of pre- α_2 -globulin. The appearance of pre- α_2 -globulin after poisoning with carbon tetrachloride in rats has been reported recently⁷. Since carbon tetrachloride is known to damage the liver, the appearance of pre- α_2 -globulin four days after the very first dose of diphtheria toxoid is probably an indication of disturbance in the liver function resulting in the excessive synthesis or secretion of the proteins of pre- α_2 -globulin mobility.

Zusammenfassung. Der Immunisierungsvorgang durch Diphtherie-Toxoid wird beim Pferd elektrophoretisch im Agar-gel verfolgt. Das Auftreten eines Prä- α_2 -Streifens wurde bald nach der ersten Toxoidgabe festgestellt.

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⁶ B. L. WAJCHENBERG, G. HOXTER, J. SEGAL, E. MATTAR, A. B. DE ULHOA-CINTRA, M. R. MONTENEGRO, and J. F. PONTER, *Gastroenterology* 30, 882 (1956).

⁷ W. G. HEIM and J. M. KERRIGAN, *Nature* 199, 1100 (1963).

The Effect of Pinealectomy and Thymectomy on the Immune Capacity of the Rat

In earlier experiments¹ it was shown that mast cell production by the lymph nodes was stimulated by pinealectomy and cortisone treatment; PAS positive cells appeared in the spleen while mast cell production in the thymus was not altered. It was assumed that the pineal body was responsible for the inhibition of mast cell production by the lymph nodes, and that, after pinealectomy, activity would go on unhampered. Since the thymus-lymph node-spleen system seems to have a decisive role in antibody production²⁻⁶, in the present experiments the effect of pinealectomy and thymectomy on the antibody production was studied in the rat.

Eighty adult rats of the Wistar strain were used in the experiment. Pinealectomy was performed without damaging the brain. The thymus was extirpated in toto, together with its capsule. If both operations were performed, pinealectomy followed the thymectomy (Table). 63

animals survived the intervention. They were immunized first with $150 \cdot 10^6$ sheep red blood cells and with $200 \cdot 10^6$ such cells as a secondary stimulus. For the hemagglutination test, blood was taken from the tail vein. The animals were studied in 2 groups, one 30 days and the other 240 days after the operations.

The Table shows the number of animals and the mean of the numbers of the last (critical) tubes showing macroscopical hemagglutination (these numbers are the logarithms to the base 2 of the reciprocals of the respective last dilutions). The standard deviation varied at about

¹ G. CSABA, M. BODOKY, and I. TÖRÖ, *Acta anat.* 61, 289 (1965).

² J. F. A. P. MILLER, *Lancet* 2, 748 (1961).

³ J. F. A. P. MILLER, *Nature* 195, 1818 (1962).

⁴ R. B. TAYLOR, *Immunology* 7, 595 (1964).

⁵ A. C. AISENBERG and B. WILKES, *J. Immunol.* 93, 75 (1964).

⁶ K. E. FICHTELIUS, G. LAURELL, and L. PHILIPPSON, *Acta path. microbiol. scand.* 51, 81 (1961).