the penis, the circumocular areas, and the ventral surface of the jaw, and had failed to regenerate on the forelegs at areas clipped for vein puncture.

The fifth and sixth dogs used as controls showed no loss of hair and normal regeneration of hair on the forelegs at areas clipped for vein puncture. The 3 experimental animals receiving the largest doses of stilbestrol revealed the maximum loss of hair in the middle of the winter of 1942-43, when the period of observation was terminated and when the 2 control dogs and several other dogs in the runs employed in other, nonhormonal experiments had a uniformly thick coat of hair.

In view of the loss of hair in male dogs following the peroral administration of stilbestrol over several months, a similar phenomenon might be anticipated in humans, particularly in the light of a recent clinical note[2] describing the loss of hair from the head of a young woman who had been given stilbestrol for several months.

[2] *J. A. M. A.*, 1943, **121**, 224.

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Acid Phosphatase Content of Nuclei of Chicken Erythrocytes.

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The recent discovery of 6 important enzymes in nuclei isolated from rat liver cells[1] has made it seem desirable to extend enzyme investigations to an entirely different type of nucleus. In this work we have investigated the acid phosphatase content of isolated chicken erythrocyte nuclei which now can be obtained in relatively undamaged condition.[2,3] The nuclei were prepared by the method of Lan and Dounce[3] from washed erythrocytes prepared from fresh defibrinated chicken blood obtained at the slaughter house. A photograph of these nuclei is shown in Fig. 1. Acid phosphatase was chosen for the investigation because it is relatively easy to determine and already has been reported in whole erythrocytes.[4,5] Moreover, it is one of the enzymes found in isolated liver cell nuclei.[1]

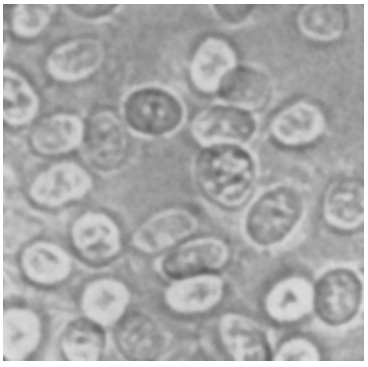

FIG. 1.

Isolated nuclei of chicken erythrocytes, oil immersion, unstained.

The method employed for the determination of the enzyme was a slight modification of Gutman and Gutman's adaptation[6] of the original alkaline phosphatase method of King and Armstrong[7] to the determination of acid

[1] Dounce, A. L., *J. Biol. Chem.*, 1943, **147**, 685.

[2] Laskowski, M., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 354.

[3] Dounce, A. L., and Lan, T. H., *Science*, 1943, **97**, 584.

[4] Roche, J., and Bullinger, E., *Enzymologia*, 1939, **7**, 278.

[5] Roche, J., *Biochem. J.*, 1931, **25**, 1724.

[6] Gutman, E. B., and Gutman, A. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 513.

[7] King, E. J., and Armstrong, A. R., *Canad. Med. Assn. J.*, 1934, **31**, 376.

phosphatase. In this method, buffered disodium phenyl phosphate is used for substrate. The buffered substrate is made by mixing equal parts of 0.22% disodium phenyl phosphate and 0.4 molar acetate buffer adjusted to give a pH of 5.0 when diluted one to two. The substrate and buffer are mixed immediately before use.

In carrying out the determinations, 0.5 cc portions of a suspension containing from 5 to 10 mg of nuclei on a dry weight basis were

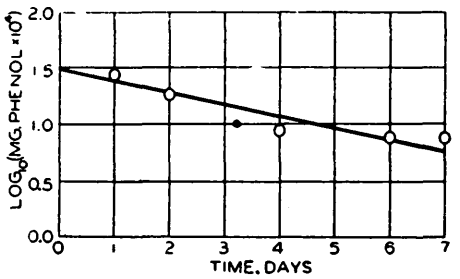

FIG. 2.

Rate of decay of acid phosphatase of chicken erythrocyte nuclei. Ordinate = $\log_{10}$ (10^4 times mg phenol liberated) per mg nuclei, dry weight, by incubation with substrate for 1 hr. Abscissa = age of preparation of nuclei in days, stored at approximately 3°C.

added to 10 cc portions of buffered substrate in test tubes, and the tubes then were placed in a thermostat bath held at 25°C and were agitated from time to time. The reaction was stopped by the addition of 5.0 cc of the diluted phenol reagent, and the test tubes were placed in the icebox for 10 to 15 minutes to allow the precipitated material to agglutinate. The precipitate was removed by filtration, the filter paper was washed once with distilled water, and then 3.5 cc of 15% sodium carbonate solution were added to the clear filtrate. The test tubes were heated in boiling water for 5 minutes to develop the color, then cooled in tap water, and finally the contents were filtered. The filter paper was washed once with distilled water, and the filtrate was diluted to 25 cc. The amount of color developed was read in a photoelectric colorimeter. Blank determinations were run at the same time on samples which had not been incubated with substrate. The readings obtained from the blank runs were subtracted from corresponding readings obtained from the incubated samples, in order to obtain values to be used in calculating the amount of phenol liberated by the enzyme from the substrate. Dilute phenol in 0.2 M acetate buffer of pH 5.0 was used in preparing standards of known concentration.*

Although the concentration of acid phosphatase found in the isolated erythrocyte nuclei was rather low (about 15 to 20 % of the concentration reported for the nuclei of normal rat liver cells),[1] its value was readily measurable. The average acid phosphatase concentration of fresh preparations of nuclei is sufficient to cause the liberation of 0.0025 to 0.0030 mg of phenol from the substrate per mg of nuclei (dry weight) per hour at 25°C and pH 5.0.

The activity of the acid phosphatase of chicken erythrocyte nuclei diminishes with time, even when the isolated nuclei are kept in the icebox at approximately 3°C, so that fresh preparations must be used if maximum activity is to be obtained. The rate of decay of enzyme activity when the nuclei are kept

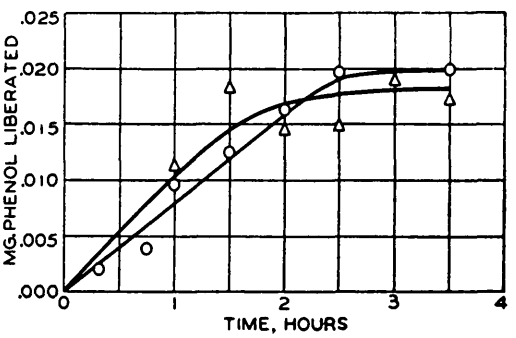

FIG. 3.

Action of acid phosphatase of chicken erythrocyte nuclei. ○ = 6.5 mg nuclei dry weight. △ = 5.2 mg nuclei, dry weight. Ordinate = mg phenol liberated from substrate by action of the enzyme. Abscissa = time of incubation of nuclei with substrate.

at about 3°C is shown in Fig. 2. The activity of the enzyme is immediately abolished if a suspension of the nuclei is boiled.

* The above procedure is the same as that which was used but not described in detail for the determination of acid phosphatase in nuclei isolated from normal rat liver,[1] except that 0.5 cc of a suspension of erythrocyte nuclei was substituted for 0.1 cc of a suspension of liver cell nuclei.

During incubation of the nuclei with the substrate, the nuclei remain in unbroken condition, since it has been found impossible so far to break them up without causing agglutination into a single mass of material. This fact may mean that the measured enzyme activity of the nuclei is somewhat lower than the true activity, since diffusion of substrate into the nuclei and diffusion of reaction products out of the nuclei may be limiting rate factors.

In the course of the determination of the acid phosphatase in isolated chicken erythrocyte nuclei, a curious phenomenon was observed, which is illustrated in the curves shown in Fig. 3. These curves show amounts of phenol liberated from the substrate by given weights of nuclei, plotted against time. The curves go up almost linearly and then flatten out rapidly at the end of a period of about 2 hours. Further incubation of the nuclei with the substrate results only in the slow uncatalyzed decomposition of the substrate. We have attempted to ascertain the cause of this abrupt loss of enzyme activity, and have finally concluded that it may be the effect of the substrate in inactivating the enzyme, although this has not been proved directly. The effect is not caused by the addition of as much as 0.04 mg of phenol to the nuclei before incubation with the substrate. Addition of 0.047 mg of phosphate buffer before addition of the substrate also caused no apparent inhibition of the enzyme. Furthermore, nuclei kept at 25°C for 2 hours and 15 minutes in the presence of buffer alone at pH 5.0 (the pH employed in the determination of the enzyme) showed no decrease in enzyme activity. This length of time is greater than the length of time necessary to reach the flat portion of the substrate decomposition-time curve.

It should be kept in mind that the nuclei of chicken erythrocytes prepared by the method employed in this work are surrounded by an extremely tenuous stroma, generally invisible except with the oil immersion objective after staining. Such a stroma also was noticed surrounding nuclei prepared by the method of Laskowski.[2] However, it is improbable that this stroma can contribute more than a minor amount to the observed enzyme activity of the preparations, since it does not appear to constitute a very appreciable fraction of the mass of the nuclear material.

We have found that the activity of washed whole chicken erythrocytes is about the same as that of the isolated nuclei.

Summary. 1. The enzyme acid-phosphatase has been determined in nuclei isolated from washed chicken erythrocytes. The activity of fresh preparations determined by the use of disodium phenyl phosphate as substrate corresponds to the liberation of about 0.0025 to 0.0030 mg of phenol from the substrate per mg of tissue per hour at 25°C. Approximately the same concentration of enzyme has been found in the washed whole erythrocytes. 2. After incubation of the nuclei with the substrate for a period of about 2 hours, the activity of the acid phosphatase quickly drops to a negligible value.