Fluorescence of Mixtures of Arterenol, Epinephrine, and Phosphate[1]

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Recent studies on arterenol, including the demonstration of its presence in the adrenal medulla (*5*), have initiated a series of attempts to estimate its concentration in biological fluids. The possible coexistence of epinephrine in these fluids necessitates parallel determination of epinephrine. In the fluorimetric method of assay chosen in this laboratory, incident light (3,650 A) from an ultraviolet source irradiates a solution containing the sample. Addition of concentrated alkali facilitates oxidation by dissolved oxygen of arterenol and epinephrine to short-lived fluorescent products. For epinephrine the

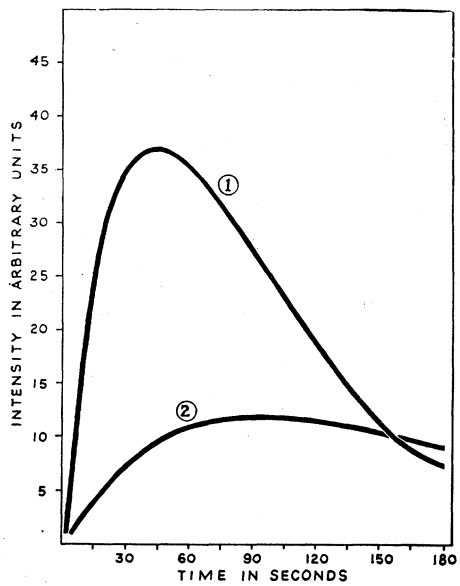

FIG. 1. Fluorescent intensity vs. time. (1)—Two μg of epinephrine in 10 ml of 9% KOH. (2)—Ten μg of arterenol in 10 ml of 9% KOH.

fluorescent substance is postulated to be 1-methyl-5,6-dihydroxyindoxyl (*1, 4*). Fluorescence of these substances is measured with a suitable recording apparatus (*3*).

Although the fluorescent spectra of arterenol and epinephrine are very similar (*3*), the maximum intensity of the arterenol fluorescence is reached at a later time than that of epinephrine, as shown in Fig. 1, which also indicates that the fluorescent intensity of arterenol is smaller than that of epinephrine by a factor of approximately 1/10. Surprisingly enough, instead of finding two fluorescent maxima when both substances were co-

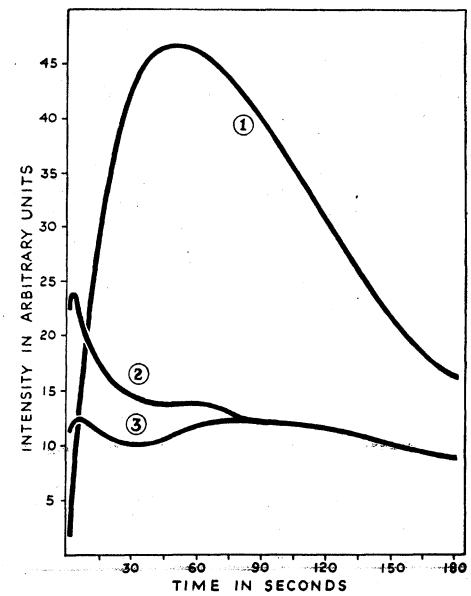

FIG. 2. Fluorescent intensity vs. time. (1)—Sum of the two curves in Fig. 1. (2)—Two μg of epinephrine and 10 μg of arterenol in 10 ml of 9% KOH. (3)—One μg of epinephrine and 10 μg of arterenol in 10 ml of 9% KOH.

existent in a test solution, arterenol and epinephrine or their respective oxidative products were found to interact to produce significantly changed curves of fluorescent intensities.

As can be seen from Fig. 2, addition of arterenol to epinephrine leads to an accelerated production of a fluorescent substance with rapid decay. Both the rapidity and the course of the reaction are related to the quantities of arterenol present in the mixture with epineph-

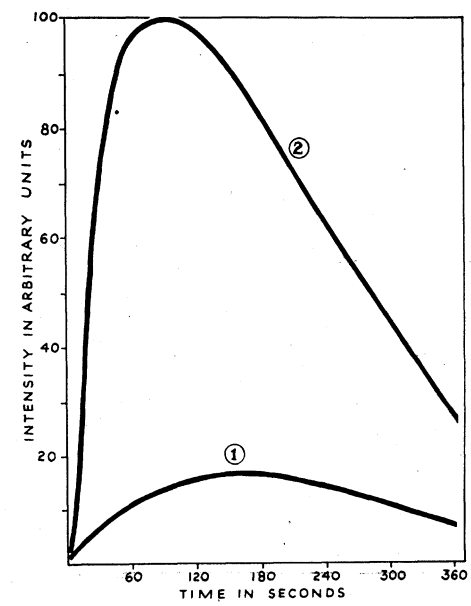

FIG. 3. Fluorescent intensity vs. time. (1)—Ten μg of arterenol in 10 ml of 9% KOH. (2)—Ten μg of arterenol plus 12 mg Na_3PO_4 in 10 ml of 9% KOH.

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rine. As little as 0.2 μg of arterenol per ml can produce this effect. It can be determined visually that the initial peak in curves 2 and 3 (Fig. 2) has the light green color of epinephrine fluorescence, whereas the secondary peak has the deeper green color of arterenol.

In the course of these studies various buffers were used. When small quantities of inorganic phosphate were combined with arterenol before the addition of hydroxide, the fluorescent maximum was increased by a factor of 6, and the time required to reach the maximum was reduced by 1/2 (Fig. 3). However, when the phosphate was combined with the hydroxide, and this mixture added to the arterenol, no phosphate effect was observed. When epinephrine and phosphate were combined, a diminution in fluorescent intensity occurred, and the time to reach the maximum was slightly reduced. A simple summation of these effects was found when arterenol, epinephrine, and phosphate were tested in solution together.

Although the explanation for the described action of arterenol on epinephrine is not at hand, it is suggested that oxidation products of arterenol may act as oxidizing agents on epinephrine. Such an assumption finds support in observations made by Falk (*2*) which describe the increased rate of epinephrine oxidation in the presence of one of its oxidation products, adrenochrome. The influence of phosphate on arterenol cannot be explained in this manner. It is assumed that the fluorescent product of arterenol contains an indole ring, as does the fluorescent substance of epinephrine, and that ring formation is accelerated in the presence of phosphate.

The physiological significance of these interrelationships is under investigation.

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Biotin Deficiency as the Causative Agent of Induced Cryptorchidism in Albino Rats

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In 1948, the egg-white injury condition (*1*) was produced in four weanling male albino rats, by feeding an experimental diet in which spray-dried egg white at a 66% level was the sole source of protein. Four additional weanling males were maintained as controls on a commercial stock diet. The testes of all animals fed the experimental diet failed to descend. Autopsy revealed these organs to be abnormally small and located in the posterior abdominal region.

In an experiment just concluded, four 30-day-old rats, in which the testes had already descended, were placed on the same experimental diet. As a control group, four additional males of the same age were also placed on the same diet in which, however, the egg white was heat-treated at 121° C for 3 hr. By the 19th day, the testes of all animals fed the unheated egg white had returned to the abdominal position. Autopsy produced the same findings as in the earlier test. Histological examination of the testis tissue showed incomplete spermatogenesis and abnormally small or completely closed tubule lumina (*3*).

That the egg-white injury condition is a biotin deficiency has been shown by Eakin *et al.* (*2*). In the advanced stages of the deficiency in this particular experiment (as in the 1948 test) there was, among other symptoms, a condition in which many of the muscles of the biotin-deficient animals were in a continuous state of tetany. This suggests that the retention of testes in the abdomen, in the one instance, and the return of these organs to the abdomen, in the other, are due to the contracture of the cremaster muscle. These data appear significant, since the growth and development of the control animals in both experiments were normal.

The results of the described exploratory tests merit further and more extensive investigation.

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