of certain histamine liberators sometimes causes heparin to appear in the blood of the animals. The presence of native heparin in the blood, however, does not necessarily coincide with clearing of a lipemia; fat clearing may occur during shock either in the presence or in the absence of detectable amounts of native heparin in the blood of dogs. In several experiments, the injection of dextran (M.W. 75,000) produced marked clearing of a heavy alimentary lipemia in the absence of heparin activity in the blood of dogs. Protamine sulfate caused the return of both visible turbidity of the plasma and of whole blood chylomicron counts to control levels after fat clearing due to shock. This occurred both in the presence and absence of significant blood heparin activity.

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Comparative Study of Epinephrine and an Epinephrine-like Substance Obtained from Autolyzing Adrenal Glands.* (20178)

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During an assay for adrenalin from minced, autolyzing adrenal glands which have been placed in dialyzing bags it was noticed that the dialysate had acquired a bluish-violet color. The pressor substance could not be precipitated from the dialysate by the addition of strong ammonium hydroxide but yielded epinephrine on acid hydrolysis. When the glands were sliced with stainless steel knives the bluish-violet color was not obtained. Suspecting contamination from the food chopper, we added various metallic salts to a purified adrenalin solution and it was found that ferric ammonium oxalate gave a comparable color. Solvents such as ether, chloroform, benzene and carbon tetrachloride failed to extract the color. In comparing the bluish-violet dialysates with Parke Davis (P.D.)† adrenalin to which ferric ammonium oxalate had been added, 10% HCl was added to both solutions; the dialysate turned colorless and the solution of adrenalin and ferric ammonium oxalate became a light yellow color. When 10% NaOH was added to the adrenalin and ferric ammonium oxalate an orange precipitate formed, but when 10% NaOH was added to the dialysate it turned pink. Both solutions contained 0.05 mg of adrenalin per ml as shown by bio-assay. P.D. adrenalin was used as the standard.

Folin(1) has shown that uric acid and Doty (2) has shown that ascorbic acid, both contained in adrenal glands, can interfere with the formation of colored compounds of epinephrine and certain metallic salts. It was found that ascorbic acid prevents the formation of the bluish-violet color of ferric ammonium oxalate and adrenalin but that uric acid neither prevents the formation of the

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† Parke Davis adrenalin.

color nor does it give the color itself as it does in Folin's method. It seemed advisable to us that if this color formation was to be made the basis of a method for the determination of adrenalin, that ascorbic acid should be prevented from interfering. It was unknown to Folin in 1912 that adrenal glands contain ascorbic acid, but Szent Gyorgy(3) found in 1928 that adrenal glands contain 600 mg of ascorbic acid per kg. Ascorbic acid also affects the formation of color in Folin's method. Edlbacher and Leuthardt (4) state that ascorbic acid and minute amounts of copper will inactivate urease. We found that the reverse was also true and that if a solution of adrenalin and ascorbic acid is heated with 0.1 g of urease but without the addition of copper for about 10 minutes the ascorbic acid will not prevent the formation of color when ferric ammonium oxalate is added.

Since the color development of epinephrine and ferric ammonium oxalate is more stable and also more sensitive than the color developed by Folin's method or Doty's method and since uric acid does not interfere in our method and ascorbic acid can be blocked, we are giving the procedure to be followed for the determination of epinephrine in adrenalin filtrates or precipitates.

Method. To a 12.5 mg sample in 250 ml of H_2O, add 2 ml of a 10% solution of ferric ammonium oxalate ($Fe(NH_4)_3(C_2O_3)_3 \cdot 6H_2O$), (stable at least one week). Shake well and let stand for 10 to 15 minutes, then read in a spectrophotometer at 555 mμ (or wavelength which is the center of extinction for the standard used. (Fig. 1). A water blank should be used. When transmittancy is plotted on semilogarithmic paper, the curve is not exactly linear except at midportion, and the greatest accuracy is between 45% and 65% transmittancy. This method is accurate from less than 1% to 5% as the greatest variation, depending upon the concentration being used. If the concentration of epinephrine is very small and the green of the reagent masks the characteristic blue color, it is advisable to use a larger sample and less of the reagent. The accuracy of a Beckman D. U. spectrophotometer varies as much as 6% at 373 mμ when compared to

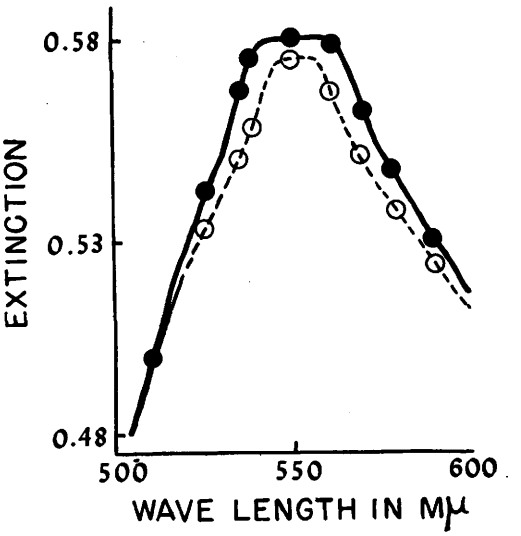

FIG. 1. Broken line represents extinction curve obtained by using P. D. (Parke Davis) adrenalin and ferric ammonium oxalate. Unbroken line represents extinction curve obtained from autolyzing adrenal glands. P. D. adrenalin curve is slightly sharper than the curve from the dialysate but both curves center at 552 mμ. Depth of the absorbing liquid was 1 cm and water blanks were used. The instrument was a Beckman model DU spectrophotometer. The P. D. adrenalin was a sample that had been in the laboratory for a considerable length of time. When a comparatively fresh (dated) sample of P. D. adrenalin was used the center of extinction fell at 575 mμ. Epinephrine samples from other sources were as follows: Armour & Co. U.S.P. suprarenalin, extra white, No. K47404 at 572 mμ; Armour & Co. U.S.P. epinephrine No. K47504 at 578 mμ; an epinephrine fresh sample prepared from adrenal glands by methods of Aldrich & Abel(8,9) at 585 mμ; Eastman No. 3097 at 572 mμ; Matheson No. 2835 at 575 mμ; and Levo-Arterenol Bitartrate $\cdot H_2O$ No. N-087-HD at 580 mμ. The center of extinction can vary by as much as 33 mμ. The data indicate that when one is using "so called" epinephrine standards, the manufacturer of the standard or the method of preparation that was used and also the shelf life of the standard should be kept in mind.

the average values of 11 other instruments, according to a U.S.P. collaborative assay(5). At 555 mμ the accuracy should be somewhat greater.

Uric acid does not interfere with the test but ascorbic acid prevents the formation of color. However, this can be prevented if 0.1 g of urease is added to the sample in about 25 cc of H_2O and heated (not boiled) for 10 minutes, then diluted to 250 ml and the reagent added. This solution can be filtered through

a fine sintered glass filter or cleared by adding $Al(OH)_3$, centrifuging and decanting.

Discussion. Apparently the ferric ammonium oxalate as such is not specific for color development since other ferric salts and oxalates together with adrenalin give similar results. Ferrous salts and oxalates when added to adrenalin give no color. Two compounds somewhat similar to epinephrine in structure, catechol and pyrogallic acid give a bluish color with ferric ammonium oxalate. However, they do not interfere with the quantitative determination. Other compounds such as tyrosine, quinhydrone, resorcinol, orcinol, phloroglucinol and ephedrine form yellow or pink colors and also do not interfere with the quantitative determination of epinephrine.

The bluish-violet color does not form in acid or basic solution outside of pH range 5.5 to 8.0; therefore the test is not applicable to blood since the usual methods use acid to precipitate the protein. In the method of Bloor and Bullen(6) for the determination of adrenalin in blood, the adrenalin reduces arseno molybdate to a colored compound. The method developed by Doty(2) was primarily for the determination of epinephrine in pharmaceutical products. In this method ferrous salts react with epinephrine in the presence of an alkaline buffer at a pH range of 8.0-8.5 to form a red-blue color having a maximum absorption at 530 mμ. A similar procedure was mentioned unfavorably by Barker, Eastland and Evers (7) who state that, "A blue color appears which is fairly stable but which fades rapidly to a purple color if too much alkali is added. The reaction did not seem sensitive enough for the estimation of traces of adrenalin." In the method of Doty(2) considerable attention has been paid to the colorimetric properties of certain compounds such as procain hydrochloride, thiourea and ascorbic acid which are often found in local anesthetic products. In regard to ascorbic acid Doty states that slight interference results from its presence in concentrations of 2 mg per ml providing the absorption is measured soon after the addition of the reagents. Among the stabilizers mentioned in this method and which have chromogenic effects, the only one which we have considered is ascorbic acid. Our method is intended for the estimation of epinephrine in adrenal glands only. In this respect it resembles the method of Folin, Cannon and Dennis(1), but with improvements in regard to handling ascorbic acid and uric acid and adaptation for use with spectrophotometers.

Summary. A bluish-violet color stable for 24 hours in a pH range of 5.5 to 8.0 results from addition of a solution of ferric ammonium oxalate to a solution of epinephrine or of suprarenal gland extracts. The transmittancy of the solution is determined at a wavelength of 555 mμ. The presence of ascorbic acid, uric acid or other naturally occurring substances found in adrenal glands does not interfere with the determination. This method is not applicable for the determination of epinephrine in blood or in drugs. The procedure is extremely simple and rapid and is capable of an accuracy of less than 1% to 5% as the greatest variation. The bluish-violet color that is obtained from minced autolyzing adrenal glands has been shown to be caused by a reaction between an epinephrine-like substance and iron products from the food chopper. A comparison of the specific extinction of various epinephrine standards and a note on the effects of dry storage is made (Fig. 1).

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