can be bound to a protein molecule has been completely developed by Klotz (7). This method is based on the law of mass action and assumes that binding occurs in a stepwise fashion, with the first mole bound being held the most firmly. The expression relating moles of small molecules bound per mole of copper-protein complex, r, with concentration of unbound ion (A) is given by

$$r = \frac{m(A)}{K + (A)}$$

Here, K is the intrinsic dissociation constant for the system, and m is the maximum number of bound ions per molecule. In order to evaluate m and K, the equation is rearranged to

$$\frac{1}{r} = \frac{K}{m}\frac{1}{(A)} + \frac{1}{m}$$

A graph of $1/r$ versus $1/(A)$ will be a straight line with the intercept on the $1/r$ axis equal to $1/m$ and the slope of the line equal to K/m.

The results of the binding studies involving histamine at *p*H values of 6.95 and 8.90 and Antistine at a *p*H of 6.95 are shown in Table 1. It was found that these binding data obeyed the law of mass action; that is, the binding increased with an increase in concentration of unbound ligand in equilibrium with the Cu(II)-proteinate.

Extrapolation of the linear plot of the reciprocals of the amount bound versus the concentration of unbound ligand yielded values for the maximum moles of ligand bound per mole of proteinate. These values were 2.75 and 20.0 for histamine at *p*H values of 6.95 and 8.90, respectively. The Cu(II)-proteinate was capable of binding a maximum of 1.74 moles of Antistine per mole of proteinate at a *p*H of 6.95.

The equilibrium constants for the first mole of ligand bound, obtained from the slope of the linear plot, were utilized to determine the free energy change for the formation of the ligand-proteinate complex. These values were -0.871 kcal and -0.910 kcal per mole for histamine at *p*H values of 6.95 and 8.90, respectively, while the corresponding value of Antistine was -0.868 kcal per mole at *p*H 6.95.

A. C. Andrews
T. D. Lyons*
Department of Chemistry,
Kansas State College, Manhattan

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1. Supported in part by grant No. E-1354 from the National Institutes of Health, U.S. Public Health Service.
2. Antistine is the commercial name for 2(N-phenyl-N-benzyl-aminomethyl)-2-imidazoline hydrochloride, which was kindly donated by the Ciba Pharmaceutical Company, Summit, N.J.
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11 July 1957

Hereditary Ovarian Tumors in Drosophila melanogaster

A recent study has been published which describes normal oogenesis in the fruit fly, *Drosophila melanogaster* (*1*). During this study ovarian tumors were observed. However, the incidence was extremely low (two tumorous chambers among 39,900 developing eggs). It was shown subsequently that ionizing radiation (4000 r of Co^{60} gamma rays) increased the incidence of tumors by 26 times.

In *Drosophila* a developing egg consists of a chamber containing 16 cells. Fifteen of the cells function as nurse cells and nourish the 16th cell (the primary oocyte). All 16 cells arise from a single cell in the germarium, which undergoes four consecutive divisions. It was postulated therefore that, in the region of the germarium where 16 cell cysts are formed, an interaction takes place between cytoplasmic substances localized in this region and the genome of the cells. The stimulated genetic material is thought next to manufacture a substance which inhibits further cytokinesis in a precise fashion. Radiation might occasionally inactivate that portion of the genome of an oogonium responsible for the production of the inhibitor. This mutation would then be passed to the progeny of the oogonium. These cells would now divide in an uncontrolled fashion and produce the observed tumorous chambers which contain hundreds to thousands of mitotically active cells.

We set out to detect mutant genes which would cause such uncontrolled cell division; but we recognized that such genes would be difficult to obtain, since they would generally produce consequences which would be lethal at an early stage in the life-cycle. From our knowledge of oogenesis, it seemed reasonable to predict that some of the genes we were looking for might be found among the 60 or so nonallelic, recessive female sterile mutants of *Drosophila melanogaster,* because uncontrolled division in egg chambers would convert developing eggs to tumors and so sterilize the fly.

We therefore obtained approximately 20 female sterile mutants and proceeded to make Feulgen whole mounts of ovaries of females homozygous for the various female sterile genes (*2*). To our amazement, the first female sterile mutant examined turned out to be a case in point, and the first ovarian preparation contained more tumors than the total we had observed from all sources up to that time. The incidence of tumors was found to increase with the age of the female. In this strain, adjacent chambers in an ovariole often fuse together. If one such chamber is tumorous and the other is normal, there will be produced a compound chamber containing normal and tumorous cells. The actively dividing tumorous cells will subsequently invade the normal tissue of the compound chamber.

This mutant which produces tumors of one tissue at one particular stage in the life-cycle is *fused* (*fu*), discovered by C. B. Bridges in 1912. It is located at 59.5 on the X-chromosome. The allele in question is spontaneous in origin, but alleles induced by x-rays or chemicals have been frequently observed. In our stock (which was obtained from the Yale collection) *fu* is balanced over M5. Females heterozygous for *fu* show no tumors. On the other hand, females homozygous for fu^{ff} (an allele of *fu* induced by formalin treatment) also show ovarian tumors. It appears that *fu,* in addition to its many other bizarre effects (*3*), produces ovarian tumors and therefore represents excellent material for further studies of the mechanism of tumorous growth.

R. C. King
R. G. Burnett
Northwestern University,
Evanston, Illinois

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26 June 1957

Fluorescence of Ethylenediamine Derivatives of Epinephrine and Norepinephrine

In 1952, Weil-Malherbe and Bone introduced a method for the chemical determination of total "epinephrinelike" substances in blood, which included separation of the catechol amines from other plasma components by adsorption chromatography and measurement of the separated fraction by fluorometry (*1*). Shortly thereafter, Persky and Ros-

ton (*2*) published a modification of the fluorometric procedure which made possible the quantitative differentiation of epinephrine and norepinephrine in mixtures, further advancing the hope of developing a practicable chemical method for the assay of these hormones in biological fluids. Subsequent attempts to apply these procedures to the quantitative measurement of epinephrine and norepinephrine in plasma, however, were not uniformly successful, and a variety of modifications have been reported (*3*). In the course of a systematic evaluation of this method in our laboratory, some sources of error have been isolated which may possibly account for some of the difficulties experienced with this procedure.

The coefficients of the equations used to calculate the relative quantities of epinephrine and norepinephrine in a mixture were determined from separate measurements of standard solutions of epinephrine and norepinephrine, a Farrand photoelectric fluorometer, model A, being used to measure the fluorescence. It was found that the ratio of fluorescence of epinephrine to norepinephrine measured in this manner was not in agreement with the values calculated from the emission spectra of the epinephrine- and norepinephrine-ethylenediamine derivatives published by Persky and Roston. The fluorescent emission spectra of these derivatives were therefore investigated.

In these experiments, a Farrand spectrofluorometer (*4*) was used for determining the spectral distribution of fluorescence. Since it was found that the norepinephrine derivative was unstable and that the rate of decay of its fluorescence was a function of the intensity of

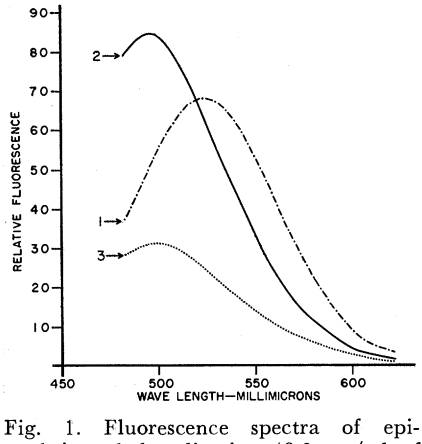

Fig. 1. Fluorescence spectra of epinephrine-ethylenediamine (0.2 μg/ml of isobutanol) and norepinephrine-ethylenediamine (0.2 μg/ml of isobutanol). Exciting wavelength is 436 mμ. Curve 1 is epinephrine; curve 2 is norepinephrine; curve 3 is the reagent blank. [Walter Reed Army Institute of Research]

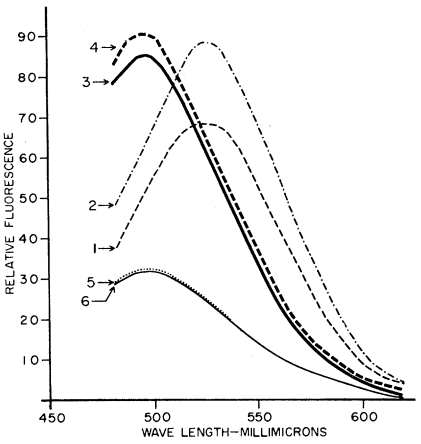

Fig. 2. Fluorescence spectra of epinephrine and norepinephrine condensed with ethylenediamine in acetic acid and in alumina-treated acetic acid. Exciting wavelength is 436 mμ. Curve 1 is epinephrine (acetic acid); curve 2 is epinephrine (alumina-treated acetic acid); curve 3 is norepinephrine (acetic acid); curve 4 is norepinephrine (alumina-treated acetic acid); curve 5 is reagent blank (acetic acid); curve 6 is reagent blank (alumina-treated acetic acid). [Walter Reed Army Institute of Research]

the exciting wave length, a Corning filter No. 3315, which passes the 436-mμ line, was used to decrease the intensity of the exciting light. The emission spectra of the ethylenediamine derivatives of epinephrine and norepinephrine (*5*) are shown in Fig. 1. The peak emission of the norepinephrine derivative is at 495 mμ, while the peak emission of the epinephrine derivative lies at 525 mμ. The ratio of fluorescence of epinephrine to norepinephrine, measured at 510 mμ, is 0.71, while the ratio measured at 600 is 4.00. These values are in agreement with the ratios determined with the Farrand model A fluorometer, Corning filters Nos. 5113 and 3389 being used in the primary, Corning filters Nos. 5433 and 3384 for the 510 mμ secondary, and Corning filter No. 2418 for the 600 mμ secondary.

An additional source of error was observed in the procedures used for determining the relative quantities of epinephrine and norepinephrine after these substances have been isolated by adsorption on alumina. A difference was observed in the fluorescence of epinephrine and norepinephrine condensed with ethylenediamine in standard acetic acid solutions as compared with the fluorescence of epinephrine and norepinephrine condensed with ethylenediamine in acetic acid which has been passed over an alumina column. It has been reported that the fluorescence of the epinephrine derivative is 100 to 150 percent greater in the acetic acid treated with alumina (*6*). In Fig. 2 the fluorescence of epinephrine and norepinephrine condensed with ethylenediamine in acetic acid is compared with the fluorescence of epinephrine and norepinephrine condensed with ethylenediamine in acetic acid which has been passed through an alumina column. It can be seen that the intensity of the fluorescence of both the epinephrine and norepinephrine derivatives is increased, the increase for the epinephrine derivative being greater than that for the norepinephrine derivative. It is also evident that there is no proportionate increase in the fluorescence of the reagent blank.

In a range of 0.02 to 0.20 μg, the average increase in the fluorescence of the epinephrine derivative measured at 510 mμ is 30 percent, while the average increase in the fluorescence of the norepinephrine derivative amounts to 9 percent. Figure 2 also indicates that the difference in the spectral distribution of the fluorescence of the derivatives prepared in acetic acid and in alumina-treated acetic acid is of a quantitative rather than a qualitative nature.

GEORGE F. MANGAN, JR.
JOHN W. MASON
Department of Neuroendocrinology, Walter Reed Army Institute of Research, Washington, D.C.

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14 June 1957

Bilirubin Glucuronide Formation in vitro; Demonstration of a Defect in Gilbert's Disease

Cole and Lathe (*1*) succeeded in isolating preparations of bilirubin which, free of protein, yielded either direct (immediate) or indirect (delayed) reactions with diazotized sulfanilic acid (van den Bergh reaction). More recently, Billing and Lathe (*2*), Schmid (*3*), and Talafant (*4*) have demonstrated that direct-reacting bilirubin is the glucuronide of bilirubin. A substance present in boiled liver extract, later shown to be uridine diphosphate glucuronic acid (UDPGA), has been found to enhance the formation of glucuronides of various receptors by rat liver homogenate (*5*). An enzyme (transferase) present in the microsomal fraction of rat liver has been shown to