structural genes do control the heavy γG-Be1 and γG-Be2 chains, it may be possible to detect recombinants among the backcross progeny of $F_1$ (BALB/c × C57BL/6) mice to C57BL/6 mice.

Herzenberg (*15*) recently reported linkage of allotypic specificities for γA ($\beta_2$A) and γG-Be1 ($\gamma_2$a) immunoglobulins. The tryptic-peptide maps of the γA and γG-Be1 heavy chains from γA and γG-Be1 myeloma immunoglobulins show vastly different patterns (*8*, *9*). Thus there appear to be three closely linked allotypic specificities at the *Asa* locus. These findings indicate that at least one complex locus takes part in the synthesis of heavy-chain subunits of immunoglobulins in mice.

The allotypic specificities of immunoglobulins in mice are the most accessible of any mammalian system for combined chemical and genetic analysis and provide useful comparative models for the analysis of data on the genetic control of human immunoglobulins where questions of linkage are more difficult to establish (*16*).

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### References and Notes

1. A "Nomenclature for Human Immunoglobulins" recommended by the World Health Organization Committee is applied here to the mouse immunoglobulins: γA (for $\beta_2$A or $\gamma_1$A); γM (for $\beta_2$M or $\gamma_1$M) and γG (for 7*S* γ or $\gamma_2$). We have further designated the three 7*S* γG-immunoglobulins as γF, γG-Be1 (Bethesda 1) and γG-Be2 (Bethesda 2) based on peptide maps of the respective myeloma heavy polypeptide chains; this notation corresponds to $\gamma_1$, $\gamma_2$a, and $\gamma_2$b characterized by Fahey, Wunderlich, and Mishell (*2*) on the basis of immunochemical differences. The Fc-fragment obtained by papain digestion is the nonantigen binding fragment (corresponding to the crystallizable fragment in rabbit antiserum). R. Ceppellini *et al.*, *Bull. World Health Org.* **30**, 447 (1964).
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6. For brevity, the five allotypic specificities Asa1, Asa2, Asa3, Asa4, and Asa5 are referred to as a1, a2, a3, a4, and a5. Similarly, the five alleles $Asa^1$, $Asa^2$, $Asa^3$, $Asa^4$, and $Asa^5$ will be referred to as $a^1$, $a^2$, $a^3$, $a^4$, and $a^5$. The prefix a1, a2, before a mouse strain indicates the allotypic specificity present.
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10. Since the results of this paper indicate that different antisera may react with the sera of the same inbred strains of mice and at the same time react with different allotypic specificities present on different immunoglobulins in these inbred mice, the notation a1′ and a1″ is used to designate these different allotypic specificities, as has been done for the rabbit (*11*) until genetic or other studies give unequivocal evidence that these are controlled by different structural genes.
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28 January 1965

## 5-Hydroxytryptamine in Single Neoplastic Mast Cells: A Microscopic Spectrofluorometric Study

Abstract. *5-Hydroxytryptamine, catecholamines, and histamine in mast cells can be distinguished by fluorescence microscopy. Microscopic spectrofluorometry can be used to estimate 5-hydroxytryptamine in single neoplastic mast cells grown in vitro, and to study the effect of reserpine on these cells.*

Treatment of various tissues with formaldehyde vapor produces fluorescent derivatives of a number of amines which have characteristic colors when examined by fluorescence microscopy. Lagunoff, Phillips, and Benditt (*1*) treated freeze-dried tissues with formaldehyde vapor to demonstrate histamine in mast cells. Falck and coworkers (*2*) treated freeze-dried tissues with formaldehyde vapor to demonstrate norepinephrine, epinephrine, and dopamine in brain and in peripheral adrenergic fibers, and these investigators attributed yellow-green fluorescence to norepinephrine, and yellow fluorescence to 5-hydroxytryptamine (5HT). Adams-Ray *et al.* (*3*) recently applied this histochemical technique to mast cells in the skin of mammals, and found in some the greenish-yellow color associated with catecholamines (*4*).

The treatment of various tissues with formaldehyde vapor thus appears to result in the formation of fluorescent derivatives of various amines (*5*). Since it may be difficult to differentiate among these derivatives visually by fluorescence microscopy, an objective histochemical technique is required. Several investigators have used microscopic fluorometry or spectrofluorometry to estimate low concentrations of endogenous compounds in tissues or single cells (*6*). Ritzén (*7*) described the application of microscopic spectrofluorometry to the identification of formaldehyde derivatives of norepinephrine and 5HT in various tissues.

To select excitation and emission wavelengths which would permit separation of the three major categories of biogenic amines associated with mast cells, the following determinations were made. The fluorescence characteristics of 5HT, norepinephrine, and histamine were determined by incubating 1.0 m*M* solutions of each amine in 20 m*M* phosphate buffer (*p*H 6.8) containing 1 percent formaldehyde, at 60°C for 2 to 4 hours. Excitation and emission spectra were determined in the Aminco-Bowman spectrofluorometer and corrected for phototube response (RCA 1P21) with the manufacturer's average spectral response curve. Fluorescence characteristics in the solid state were determined by drying 0.01 ml of 5 m*M* amine in 1 percent crystalline ovalbumin solution on a microscope slide, heating the residue in the presence of paraformaldehyde (polymerized formaldehyde) at 80°C for 1 hour, and examining the material in the microspectrofluorometer (*8*). The results obtained are summarized in Table 1. Values for norepinephrine in solution varied widely with *p*H, but in the solid state the emission maximum was very close to the value reported by Corrodi and Hillarp (*5*). The fluorescence of the 5HT derivative did not vary in the aqueous and dry states, and was close to that previously reported (*5*). The histamine derivative showed an increase in excitation and emission maxima in the solid state, in comparison with aqueous solution; these values are similar to those obtained by Lagunoff (*9*) under somewhat different conditions.

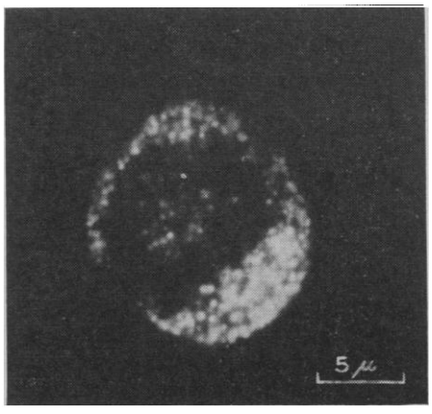


Fig. 1. Fluorescence photomicrograph of a murine mastocytoma cell grown in vitro, and treated with formaldehyde vapor. The fluorescence characteristics as measured in the microspectrofluorometer are identical with those of the formaldehyde derivative of 5HT. Excitation with mercury burner and Schott BG12, 2 mm, through dark-field condenser. Barrier filter OG-1, Adox KB-14 film.

Neoplastic mast cells (derived from the Dunn-Potter strain of mouse mastocytoma) were grown in vitro, harvested, and assayed for 5HT as described by Carlini, Fischer, and Giarman (*10*). These cells were prepared for fluorescence microscopy by centrifuging them from 5 ml of medium at 1000*g* for 5 minutes, decanting the supernatant medium, and gently depositing a small portion of the pellet on a microscope slide. The preparation was dried in air for 10 minutes, and then heated in air (control) or in the presence of paraformaldehyde at 80°C for 1 hour. The slides were covered with mineral oil and cover slips applied. Figure 1 shows a fluorescence photomicrograph of a cell from a clone having large concentrations of 5HT; the fluorescence characteristics of this cell were identical with those of the formaldehyde derivative of 5HT. Individual granules were not always as distinct as in this photograph. No fluorescence could be seen in control cells heated without formaldehyde.

Cells of a clone which normally contained amounts of 5HT in the order of 0.009 μmole (1.5 μg) per million cells were incubated in the culture medium with reserpine (1.0 μ*M*) for periods up to 24 hours. Controls consisted of cells from the same inoculum, incubated in reserpine-free culture medium. The 5HT was determined by fluorometric assay (*10*), and single cells were measured in the microspectrofluorometer with 436-mμ exciting light and a 540-mμ barrier filter (*8*). Figure 2 shows a comparison of the distribution of fluorescence of individual cells in 52 control cells and 50 cells exposed to reserpine for 1 hour. On observation with the fluorescence microscope, and on measurement of groups of cells, the fluorescence of single cells varied greatly (see the control cells in Fig. 2). The fluorometric assay for 5HT of these cells was 1.56 μg per million cells. After exposure to reserpine (1.0 μ*M*) for 1 hour the 5HT was reduced to 0.64 μg per million cells, and the fluorescence of individual cells tended to cluster at very low values. A few cells retained very large fluorescence values, which was also observed visually with the fluorescence microscope. Fluorescent cytoplasmic granules (Fig. 1), which were numerous in most control cells, became sparse after treatment with reserpine. Exposure to reserpine for longer periods caused a more complete depletion of 5HT, and a greater proportion of nonfluorescent cells. These observations suggest a differential susceptibility to the action of reserpine in this population of cells.

The fluorescence observed in these neoplastic mast cells in culture is believed to be due largely to the 5HT-formaldehyde derivative for the following reasons. (i) The concentration of 5HT in these cells far exceeds that of histamine, the only other compound present likely to form a fluorescent derivative under these conditions. (ii) Depletion of 5HT by reserpine is paralleled by a reduction in the distribution of fluorescence of individual cells. (iii) The fluorescence observed in single cells has similar excitation and emission maxima to the formaldehyde derivative of 5HT, measured in solution and in the solid state. (iv) Incubation of a clone of cells low in endogenous 5HT in a medium containing 5HT results in the development of fluorescence identical to that observed in cells of a clone with normally high concentrations of 5HT.

It is our impression that the color

Table 1. Fluorescence characteristics of formaldehyde derivatives of norepinephrine, 5HT, and histamine.

| Amine | Excitation maxima (mμ) | | Emission maxima (mμ) | |
|---|---|---|---|---|
| | Aqueous | Dry protein film | Aqueous | Dry protein film |
| Norepinephrine | 370 | 436* | 450 | 480–500 |
| 5HT | 425, 470 | 436* | 535 | 540 |
| Histamine | 340 | 365* | 400 | 440 |

* Excitation wavelengths were restricted to the 365, 405, and 436 mμ Hg lines—therefore, these are not exact excitation maxima.

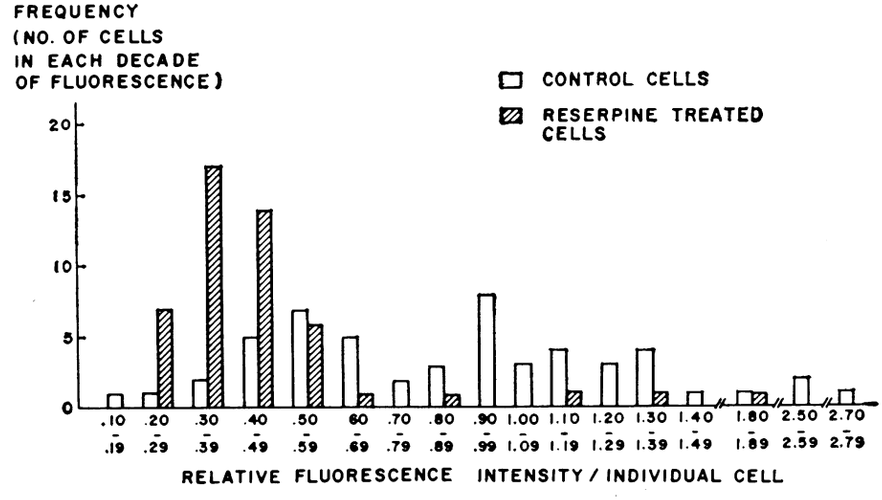


Fig. 2. Distribution of relative fluorescence intensity of 52 control cells (1.56 μg of 5HT per million cells by fluorometric assay) and 50 cells treated with reserpine (1.0 μ*M*) for 1 hour (0.64 μg of 5HT per million cells). Cells are grouped in decades of relative fluorescence intensity with ranges of 0.10 units each. Excitation was at 436 mμ and emission measured at 540 mμ (*8*).

perceived visually in fluorescence microscopy is insufficient for positive identification of a product. The introduction of interference filters reduces the artifact of unequal color perception by the eye and provides increased selectivity without serious loss in sensitivity. Such a maneuver is convenient for separating the fluorescence of the formaldehyde derivatives of norepinephrine and 5HT in tissues where they occur together, such as the pineal body (*11*), and for separating the fluorescence of the formaldehyde derivative of histamine from the other derivatives. Photometric measurement of emitted fluorescent light permits a quantitative estimate of identifiable biogenic amines seen in fluorescence microscopy.

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4. The formaldehyde condensation products are believed to be the following: norepinephrine, 1,6,7-trihydroxy-3,4-dihydro isoquinoline (*5*); 5HT, 6-hydroxy-3,4-dihydro-β-carboline (*5*); histamine, tetrahydropyrido-3,4-iminazole [see *1*, and H. H. Dale and H. W. Dudley, *J. Pharm. Exptl. Therap.* **18**, 103 (1921)].
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4 February 1965

## Epinephrine-Induced Normalization of Lipid Metabolism in Adrenalectomized Rats

Abstract. *In adrenalectomized rats given adequate supportive therapy with sodium chloride and hydrocortisone, lipemia is markedly elevated after a triglyceride meal. This defect can be corrected by the administration of epinephrine.*

Recently we observed (*1*) that patients with Addison's disease exhibited postprandial delay in the rate of decrease in the concentration of plasma triglyceride, even though these patients were adequately supplied with hydrocortisone and desoxycorticosterone acetate. A similar delay also occurs in subjects with coronary artery disease (*2*) and in healthy subjects particularly prone to coronary artery disease (*3*). This prolonged elevation of the concentration of plasma triglyceride is surprising since absorption of fat is deficient in both man (*4*) and animals (*5*) suffering from untreated adrenal insufficiency. Our observation suggested that patients with Addison's disease, who apparently received satisfactory treatment, still lacked some adrenal hormone(s) (other than hydrocortisone and desoxycorticosterone acetate) associated with adequate lipid transport or disposition. To investigate this possibility we subjected adrenalectomized rats to a series of studies.

In the first study, four groups of adult male rats (Long-Evans strain), weighing approximately 300 g each, were adrenalectomized. The first group of ten adrenalectomized rats received subcutaneous injections of 0.075 mg of epinephrine in oil at approximately 9:00 a.m. and again at 4:00 p.m.; the second group of 12 received 0.5 mg of hydrocortisone subcutaneously twice each day at similar times; the third group of 13 received both drugs subcutaneously twice each day; while the fourth group of 6 rats received no drugs. All adrenalectomized rats were given 0.8 percent NaCl to drink. Seventy-two hours after adrenalectomy all rats were given 3 ml of cottonseed oil by stomach tube, the abdominal segment of each thoracic duct was cannulated by a previously described technique (*6*), and intestinal lymph from each rat was collected separately for 3 hours and analyzed for triglyceride content (*7*). For control purposes, sham operations were performed on 17 rats; 72 hours later, after 12 of these rats had received 3 ml of oil, the lymph ducts of all 17 were cannulated, and lymph was collected for 3 hours and analyzed for triglyceride (*7*). During the studies all rats were housed in a quiet room isolated from ordinary laboratory activity except for the time during which samples of lymph or blood were collected.

In the second study three groups of adrenalectomized rats, which were also given 0.8 percent NaCl to drink, were injected with epinephrine (0.075 mg twice each day), hydrocortisone (0.5 mg twice each day), or both. A fourth group of adrenalectomized rats received no drugs. For control purposes four groups of rats were given similar treatment after sham operations. All