which the intensity ratio is changed. These graphs were obtained from the same animal and with the same electrode locations, except as noted in the legends. Similar results have been obtained with several orientations of the electrode array within auditory area AI, and with click No. 1 both ipsilateral and contralateral to the recording electrodes.

These data suggest that the angular location of auditory stimuli may be represented in the auditory cortex of one hemisphere by means of a place principle.

PAUL D. COLEMAN
Institute for Applied Experimental Psychology, Tufts University, Medford, Massachusetts

References and Notes

1. J. V. Bekesy, *Physik. Z.* 31, 824, 835 (1930); E. G. Boring, *Am. J. Psychol.* 37, 157 (1926); T. J. Bowlker, *Phil. Mag.* 15, 318 (1908); L. A. Jeffress, *J. Comp. and Physiol. Psychol.* 41, 35 (1948); H. Pieron, *The Sensations* (Yale Univ. Press, New Haven, Conn., 1952).
2. M. R. Rosenzweig, *J. Comp. and Physiol. Psychol.* 47, 269 (1954).
3. The work reported here was supported by National Science Foundation grant No. G-3850.
4. Single elements sensitive to the apparent location of auditory stimuli have been seen in the olivary complex by Robert Galambos.

9 February 1959

Nature of Colchicine Resistance in Golden Hamster

Abstract. The unresponsiveness of the golden hamster to colchicine has been shown to be the result of a resistance of individual cells to the drug. This resistance makes it possible to use hamsters with heterotransplanted tumors for testing the in vivo effects of colchicine on the tumors, with no toxic host reactions.

Orsini and Pansky (*1*) have reported a resistance on the part of the golden hamster to the toxic and mitotic inhibitory effects of colchicine. In a study of lethal effects, Turbyfill and Soderwall (*2*) showed that this resistance was at least 100 times greater in the hamster than in other animals. Whether this unresponsiveness is the result of a systemic inactivation of the drug or the result of a resistance of the individual cells has not been determined. The experiments described in this report indicate that the golden hamster has a true cellular resistance to the action of colchicine.

The first experiment was undertaken to determine whether heterologous tissues grown in hamsters were sensitive to colchicine. Three transplanted human testicular tumors (*3*), a teratocarcinoma of the ovary of a mouse (*4*), and a spontaneous anaplastic hamster carcinoma (*5*) were maintained in the cheek pouches of cortisone-conditioned weanling golden hamsters, as described previously (*3, 6*). Experimental animals bearing tumors of approximately the same size received intraperitoneal colchicine (*6*) in dosages listed in Table 1, while tumor-bearing controls received no treatment. The tumors and a piece of duodenum were removed 18 hours later and examined microscopically. By means of an ocular reticule, the mitoses in 40 areas of constant size were counted in each tumor. Likewise, the mitoses were counted in 40 of the host's intestinal crypts, which were considered to be similar to each other in size and shape.

In a second experiment to determine the effect of colchicine upon isolated cells in vitro, we grew embryonic hamster tissue in plasma clot and human cells of the D-189 strain (Mavar) (*7*) on glass. The growth medium was made up of calf serum, chick embryo extract, and Hanks balanced salt solution (20:10:70), to which Eagle's basal medium concentrates, penicillin, and streptomycin were added. When suitable growth was obtained, 0.2 ml of serially diluted colchicine in balanced salt solution was added to the cultures (the final concentrations are listed in Table 2). Control cultures received only balanced salt solution. Six hours later they were fixed in Bouin's fluid and stained. At least 200 consecutive human and 400 hamster mitoses were differentially counted for each concentration of colchicine. The results were expressed as the percentage of mitoses which had ball-like configurations with no evident spindles typical of colchicine-induced metaphase arrests.

Table 1. Effects of colchicine on heterologous and homologous tissues grafted in the hamster. All values represent the average number of mitoses per unit area per animal studied (see text), plus or minus one standard deviation. Number of animals in parentheses.

Tissue	Control	No. of mitoses Dosage*			
		0.05 mg	0.1 mg	0.25 mg	1.0 mg
Pitt 89 (human) choriocarcinoma)	46 ± 12 (11)	112 ± 97 (8)	569 ± 154 (7)	540 ± 79 (5)	540 (2)
Pitt 61 (human embryonal carcinoma)	45 ± 8 (6)		104 ± 16 (4)	493 ± 42 (4)	
Deac 3 (human embryonal carcinoma)	63 ± 29 (16)	64 ± 12 (5)	175 ± 81 (6)	574 ± 42 (7)	
Mouse ovarian teratoma	64 ± 12 (6)		262 ± 183 (5)	744 ± 89 (4)	
Hamster carcinoma	13 ± 4 (8)			14 ± 2 (10)	14 ± 7 (9)
Hamster intestinal crypts	62 ± 15 (16)	69 ± 14 (10)	63 ± 12 (19)	57 ± 12 (17)	64 ± 10 (7)

* Doses are of colchicine (1 mg/2 cm³) per 100 g of body weight.

Table 2. Effects of colchicine upon human and hamster cells in vitro. Values represent the percentage of mitoses with the configuration of colchicine-induced "arrests" (see text).

Final concentration	Human strain D-189 (Mavar) cells	Hamster embryonic cells
(Control)	6	7
$10^{-9}M$ colchicine	16	
$10^{-8}M$ colchicine	36	
$10^{-7}M$ colchicine	99	5
$10^{-6}M$ colchicine	100	36
$10^{-5}M$ colchicine		85
$10^{-4}M$ colchicine		95
$10^{-3}M$ colchicine		96

The results of mitotic counts for the in vivo experiments listed in Table 1 indicate that colchicine had no effect on the mitotic index (the number of mitoses in a given area) of the hamster intestinal crypts or of the spontaneous hamster carcinoma within the dosage range used. There was a striking increase, however, in the mitotic index of heterotransplanted tissues of human and mouse origin, especially at the higher dosages employed. These effects generally began to appear at the 0.1 mg per 100 gram body-weight level but occasionally were noticed at the 0.05 mg per 100 gram level. Cells arrested in vivo displayed mitoses typical of colchicine treatment (*8*).

No abnormalities attributable to colchicine could be detected on gross examination in the 65 hamsters treated at 0.25- and 1.0-mg/100 g levels. This was in contrast to findings for a group of 21 C_3H mice treated with the same doses of colchicine on a weight-for-weight basis. After 18 hours four mice died and the remainder had humped backs, ruffled fur, and marked diarrhea.

The results of in vitro studies (Table 2) indicated that hamster cells had at least a 100-fold greater resistance to colchicine than human cells. A few normal metaphases viewed on end were indistinguishable from colchicine-induced metaphases, and this accounted for the "arrests" noted in the control group. Many normal anaphases and telophases could be found in the hamster tissues at concentrations of colchicine as great as $10^{-5}M$, indicating that in spite of the large percentage of arrests found, considerable resistance was maintained at this high level.

This study of human, mouse, and hamster tissues, both in vivo and in vitro, demonstrated that normal adult, neoplastic, and embryonic hamster tissues have a cellular type of resistance to colchicine that is at least 100 times greater than that of human cells. This degree of resistance for hamster cells in vitro was similar to that previously reported in in vivo toxicity experiments in which the hamster was compared with other mammals (*2*).

The existence of a cellular resistance to colchicine in the hamster will make possible investigation of the effects of high and long-continued doses of colchicine on heterotransplanted tissues, embryonic or neoplastic, with no toxic host reactions. This resistance is an example of a genetically transmitted tolerance, and, if gene-controlled, could be used as a marker in transformation studies upon mammalian cells in vitro (*9*).

A. Rees Midgley
Barry Pierce
Frank J. Dixon

Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

References and Notes

1. M. W. Orsini and B. Pansky, *Science* 115, 88 (1952).
2. C. L. Turbyfill and A. L. Soderwall, *ibid.* 126, 749 (1957).
3. B. Pierce, F. J. Dixon, E. Verney, *Cancer Research* 17, 134 (1957); *ibid.* 18, 204 (1958).
4. E. Fekete and M. A. Ferrigno, *ibid.* 12, 438 (1952).
5. A. R. Midgley, B. Pierce, F. J. Dixon, unpublished data.
6. The cortisone acetate and colchicine used in these experiments were supplied in generous amounts by Dr. F. K. Heath, of Merck, Sharp and Dohme and Co., and by Dr. G. W. Irwin, of the Lilly Laboratory for Clinical Research, respectively.
7. Strain D-189 (Mavar) cells were supplied through the courtesy of Dr. J. Leighton.
8. O. J. Eigsti and P. Dustin, Jr., *Colchicine—in Agriculture, Medicine, Biology, and Chemistry* (Iowa State College Press, Ames, 1957).
9. This report is reprint No. 196 from the Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. The investigation was supported in part by the U.S. Public Health Service, grant No. CY3297(C1).

24 February 1959

Adsorption of Antibody in vitro and Magnitude of the Schultz-Dale Reaction of Guinea-Pig Ileum

Abstract. Studies show that the adsorption of rabbit antiovalbumin-I^{131} on guinea-pig ileum conforms to a Langmuirian isotherm, and that the physiological response to challenge with antigen depends upon the amount of antibody bound to the tissue.

Recently, Germuth and McKinnon (*1*) showed that antigen-antibody complexes, formed in antigen excess, would produce gross anaphylaxis of varying degrees of severity in the normal guinea pig, and Trapani, Garvey, and Campbell (*2*) demonstrated a quantitative relationship between the antigen-antibody ratio of the complex and the degree of the Schultz-Dale response.

This report concerns the quantitative relationship between the antibody content of the tissue and the magnitude of the specific response. Since active immunization, or even passive transfer of antibodies, could not be expected to provide a reproducible degree of immunological sensitivity, a method of sensitizing tissues in vitro was investigated (*3*). Although sensitization of isolated tissues by exposure to crude antisera has been reported (*4*) we do not know of any studies in which an attempt was made to detect whether antibody was actually adsorbed on the tissue, or of data giving a quantitative relationship between the concentration of antibody bound to the tissue and the magnitude of the Schultz-Dale response.

The present report (*5*) shows that the I^{131}-labeled gamma-globulin fraction of rabbit antiovalbumin can be adsorbed by guinea-pig ileum in proportion to the protein concentration in the *bulk phase* and that the response of the muscle to antigen is a function of the amount of antibody remaining on the tissue.

The antigen used in these studies was four-times-crystallized ovalbumin prepared by the method of Keckwick and Cannan (*6*). The antiserum was prepared by injecting rabbits with ovalbumin for 6 weeks, in accordance with an injection schedule not yet published (*7*). Animals were bled by cardiac puncture, the serum was separated, and the gamma globulin was prepared by reprecipitating the antiserum four times in the presence of ⅓-saturated ammonium sulfate. The preparation was dialyzed against repeatedly changed 1-percent NaCl and dried from the frozen state. The antibody content of the soluble protein was 32.5 percent, as determined by the quantitative precipitin technique; hence, it is likely that the preparation contained other antibodies as well as normal globulins. Antiovalbumin-I^{131} was prepared from a portion of this material by the method of Hughes and Straessle (*8*). The material employed in the studies discussed in section 1, below, contained 0.01 mole of iodine per mole of gamma globulin and had a specific activity of 0.35 μc/mg gamma globulin.

1) To determine the effect of I^{131} gamma globulin concentration and washing upon the residual radioactivity of the tissue, ileal strips measuring 2.5 cm in length when relaxed were prepared from intestines of normal male guinea pigs (350–400 g) according to the method of Feigen and Campbell (*9*). These were immersed in muscle baths, constructed from sintered glass filters, having a capacity of 30 ml, each

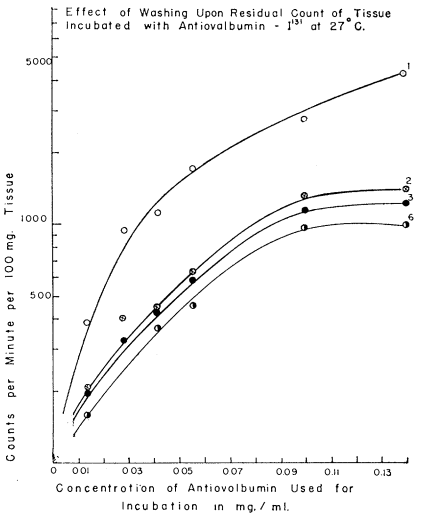

Fig. 1. Residual radioactivity on strips of normal-guinea-pig ileum incubated in various concentrations of an I^{131}-labeled gamma-globulin fraction of rabbit antiovalbumin. Curves 1, 2, 3, and 6 represent residual radioactivity after the first, second, third, and sixth washings.

containing a different concentration of I^{131}-labeled gamma globulin, ranging from 0.014 to 0.140 mg/ml. All tissues were incubated in 30 ml of Tyrode solution for 60 minutes at 27°C and then washed with 30-ml portions of fresh Tyrode solution at 27°C. The radioactivity was measured in a well-type crystal (NaI) scintillation counter. The counting efficiency with an I^{131} standard was 33.3 percent. The tissue was mounted on a glass rod and was immersed in a tube contained in the well of the scintillation counter. The radioactivity of the system was measured in the presence and in the absence of tissue, and the residual radioactivity on the tissue was obtained from the difference in the measurements. The washing and counting operations were carried out for six complete washing cycles. The results, presented in Fig. 1, are mean values for two strips tested at each concentration.

Since, as is shown in Fig. 1, the residual radioactivity of a tissue appears to bear a constant relationship to the concentration of I^{131}-labeled gamma globulin used in the original incubation, and since the radioactivity is not greatly reduced between the second and sixth washings, it appeared possible to test the data of curve 3 for conformance to the Langmuir adsorption isotherm. A convenient form of Langmuir's equation is

$$C/a = (1/\alpha\beta) + (C/\beta) \qquad (1)$$

in which C is the concentration of antiovalbumin in milligrams per milliliter, a is the amount (in milligrams) of adsorbate taken up by 100 mg of tissue, and α and β are constants. Accord-