pure lymphocytes were obtained by the method described by Terasaki *et al.* (*6*). Lymphoagglutination and hemagglutination tube tests were made with 0.05 ml of cell suspension and 0.2 ml of antisera in fourfold dilutions. Tubes were incubated at room temperature for 90 minutes. Degree of agglutination was determined by examining a drop of the mixture on a glass slide with a light microscope. Red cells and lymphocytes were tested separately at approximately the same concentration (30,000 per cubic millimeter), allowing titer comparisons. Serum dilutions and cell suspensions were made with Hanks' balanced salt solution.

The results of these tests are summarized in Table 1. The findings clearly demonstrate that chicken erythrocytes have antigens which the lymphocytes do not have. Iso-antisera of the *A*, *D*, and *L* blood group systems discriminate by agglutinating only red cells. Further confirmation of red cell specificity was obtained by absorption tests whereby the lymphocytes were found not to remove the *A*, *D*, and *L* antibodies. In contrast, the *B* and *C* system reagents agglutinated both lymphocytes and red cells. No differences between the two types of cells, with respect to titer, were observed. Since the *B* locus is known to be a histocompatibility locus (*7*), these findings would suggest that the *C* locus may also have histocompatibility properties.

Efforts were also made to produce agglutinins for lymphocytes which would be distinguishable from red cell agglutinins. In order to confer tolerance to the erythrocyte antigens, 23 newly hatched chicks were injected with 0.5 ml of 30 percent red blood cells from four adult birds. Red cells were obtained by filtering the lower layer of centrifuged blood three times through glass wool, following a method described by Billingham *et al.* (*8*). No leukocytes were found in any of several samples examined with a microscope. Red cells of the donor fowl were known to have a specific antigen (designated B_3) of the *B* system which the recipients' cells did not have. The 23 test chicks, and eight control chicks, not injected at hatching, later received two 8 by 10 mm full-thickness skin grafts and two or three subcutaneous injections of peripheral leukocytes and spleen cells from the original red cell donors or other birds having the B_3 red cell antigen. Nineteen test chicks and all control chicks received an additional skin graft from a B-locus compatible donor. Eleven test chicks and four control chicks were first injected with leukocytes and spleen cells at 17 days and grafted at 24 days after hatching; the remainder were grafted at 17 days and injected with leukocytes and spleen cells for the first time at 36 days after hatching. Serum from each chick was tested for lymphoagglutinin and hemagglutinin titer 2 or 3 weeks after the last injection.

Table 1. Results of lymphoagglutination and hemagglutination tests with antisera specific for five blood group systems.

System	No. of antisera tested	No. of antigens tested	Blood cells containing antigens
A	4	2	Red only
B	7	4	Red, white
C	2	1	Red, white
D	1	1	Red only
L	1	1	Red only

All attempts to produce iso-antibodies specific for lymphocytes were unsuccessful. The injection of red blood cells shortly after hatching markedly inhibited the capacity of the chicks to produce agglutinins for both erythrocytes and lymphocytes. Only four of the 23 test chicks produced detectable antibodies. These were demonstrable only with undiluted serum; they agglutinated both types of cells and were specific for the B_3 antigen. All of the eight control birds displayed agglutinins of higher titer (dilutions of 1 : 4 to 1 : 64). Tolerance to skin grafts was not induced in any of the chicks. All grafts from birds having the B_3 antigen were rejected within 6 to 10 days after grafting. That none of the chicks produced agglutinins specific only for lymphocytes suggests that the *B* antigens of the erythrocytes may have all the determinant groups present in the *B* antigens of the leukocytes. The results also indicate that this population does not have alleles segregating at other loci which control the formation of antigens peculiar to leukocytes. These inferences, however, depend on the validity of the assumption that the red cell suspension injected at hatching was either leukocyte-free or contained an insufficient number of leukocytes to confer humoral tolerance.

LOUIS W. SCHIERMAN
ARNE W. NORDSKOG
Department of Poultry Science, Iowa State University, Ames

24 AUGUST 1962

References and Notes

1. P. B. Medawar, "Iso-antigens," in *Biological Problems of Grafting*, F. Albert and P. B. Medawar, Eds. (Thomas, Springfield, Ill., 1959).
2. R. L. Walford, *Leucocyte Antigens and Antibodies* (Grune and Stratton, New York, 1960).
3. O. Stark, V. Kren, B. Frenzl, R. Brdicka, *Folia Biol. (Prague)* **7**, 243 (1961).
4. This work was supported in part by the National Science Foundation (grant No. G-8887). This report is journal paper No. J-4298 of the Iowa Agricultural and Home Economics Experiment Station, Ames, project No. 1039.
5. W. E. Briles, W. H. McGibbon, M. R. Irwin, *Genetics* **35**, 633 (1950); W. E. Briles, C. O. Briles, J. H. Quisenberry, *Poultry Sci.* **29**, 750 (1950); W. E. Briles, *ibid.* **30**, 907 (1951); D. G. Gilmour, *Genetics* **44**, 14 (1959).
6. P. I. Terasaki, J. A. Cannon, W. P. Longmire, Jr., *Proc. Soc. Exptl. Biol. Med.* **102**, 280 (1959).
7. L. W. Schierman and A. W. Nordskog, *Science* **134**, 1008 (1961).
8. R. E. Billingham, L. Brent, P. B. Medawar, *Phil. Trans. Roy. Soc. London Ser. B*, **239**, 357 (1956).

11 June 1962

Neurochemical Differences between Two Strains of Mice

Abstract. Microanalytic determinations of serotonin and norepinephrine were done on dissected portions of brain consisting of diencephalon, mesencephalon, and pons in two strains of mice, C57 bl/10 and BALB/c. The BALB/c strain had 1.34 μg of serotonin per gram of dissected brain, and the C57 bl/10 had 1.07 μg/g. This difference was statistically significant ($P < 0.01$). The levels of norepinephrine were not significantly different in the two strains ($P > 0.25$).

Over the past several decades there have been several published accounts of differences in the behavior of various strains of mice. In particular, the C57 bl/10 and BALB/c have been carefully investigated (*1*) with consistently similar results which may be summarized as follows. The C57 bl/10 show more exploratory activity, less emotionality as measured by the open-field defecation test, and superiority in fighting in comparison with the BALB/c strain. Because of these repeatedly demonstrated differences in behavioral parameters, it was of interest to note Caspari's report (*2*) of differences in total, pooled brain serotonin (5-HT) levels, with the C57 bl/10 having 1.162 μg/g of brain tissue and the BALB/c strain having 0.995 μg/g. However, as Caspari notes, the BALB/c strain has a heavier brain, and because serotonin is selectively concentrated in those structures usually grouped as the limbic system, the amount of serotonin determined in total brain and then expressed in terms of micrograms per gram may be misleading.

Because of the differential in exploratory activity, it was also felt that some of the present controversy (*3*) regarding the importance of serotonin vs. norepinephrine release and sedation might be profitably explored by looking for differences between serotonin and norepinephrine levels in these two strains. With this in mind, it was decided to microanalytically determine serotonin and norepinephrine levels in that portion of the brain known to contain the greatest amounts of these two amines found in total brain.

C57 bl/10 and BALB/c male mice were obtained from the Bar Harbor Laboratories. Age at time of sacrifice varied from 7 to 10 weeks. All determinations were paired—that is, for each specimen from a BALB/c animal there was a corresponding specimen from a C57 bl/10 animal, so that in any given experiment the age of the mice from each strain, as well as other variables, was the same. All animals were housed in groups of five of the same strain. Sacrifice was by decapitation. Killing times were noted and found not to differ significantly. Brain dissection was performed as follows. The cerebellar lobes, medulla, and cerebral hemispheres were removed. All brain tissue lateral to the optic tracts and anterior to the optic chiasm was dissected away. A piece of tissue consisting of diencephalon, mesencephalon, and pons remained, and, on this portion of the tissue, determinations of the two compounds were done by the microanalytic technique as described by Kuntzman *et al.* (*4*). All assays were performed using a single blind method.

Mean values, animals used, and brain weights are outlined in Table 1. Statistical analysis of the data was as follows. For both serotonin and norepinephrine values, the error mean square in a two-way mixed-model analysis of variance was partitioned by strains and found to have significantly different components in the two strains. Both for norepinephrine and serotonin the BALB/c's were found to have a greater variance, with the P values for the significance of differences between variances being 0.01 for norepinephrine and less than 0.01 for serotonin. Because of the different variances the two-way analysis of variance approach was invalid and less powerful methods had to be employed. One-way analyses of variance were carried out on experiments, separated for each strain, and then paired t-tests were employed to test strain differences. Here it was noted that the differences in serotonin levels for the two strains were significantly different ($P < 0.01$), whereas norepinephrine levels did not differ significantly ($P > 0.25$). A t-test indicates the heavier mean weight of the BALB/c specimens is statistically significant ($P < .025$). To rule out the possibility that in some way being housed in a group might be of importance, five animals from each strain were caged separately for 3 weeks. The mean serotonin levels on these animals were 1.35 μg/g for the BALB/c and 1.07 μg/g for the C57 bl/10, thus suggesting that differences are not due to some housing effect such as a different incidence of fighting within strains.

Table 1. Mean values for serotonin and norepinephrine in dissected portion of brains of mice.

Strain	Number	Amount (±S.E.M.*μg/g)
	Serotonin	
C57 Bl/10	19	1.07 ± .037
Balb/c	19	1.34 ± .046
	Norepinephrine†	
C57 Bl/10	25	0.70 ± .047
Balb/c	25	0.73 ± .043
	Weight‡ (mg)	
C57 Bl/10	45	140.7 ± 1.69
Balb/c	45	146.4 ± 1.56

* Standard error of the mean. † The pooled estimates of within experiment variances for norepinephrine are .0606 and .0083 for the Balb/c and C57 Bl/10 strains, respectively. These different dispersions are not apparent in the nearly equal standard errors of the means computed for the total sample. ‡ Weight of dissected portion of brain.

The meaning of these findings is not clear at the present time, but the many clinical studies indicating the effectiveness of the monoamine oxidase inhibitors (which elevate brain levels of serotonin and norepinephrine) in the treatment of depression; the sedation which accompanies the depletion of brain serotonin and norepinephrine by reserpine, and animal experiments (*5*) all suggest that these brain amines may be related in some way to behavioral measures. Attempts to elucidate how these amines are related to behavior, in the past, have in good part been dependent upon the induction of changes in their levels by the use of drugs with greater or lesser specificity of action. A more naturalistic difference such as is noted to occur in these two strains may lend itself easily to various experimental approaches.

In general, it is felt that not only may absolute levels of these substances be important but that in addition differences in rates of release or intracellular localization or both, of serotonin and norepinephrine may be relevant parameters for the understanding of the function of these compounds in the central nervous system (*6*). Our observations, of course, offer no answer to any of these problems but, again, the noted differences between these two strains may offer an important investigational tool free of artifacts introduced when one uses drugs to induce changes. It seems particularly relevant here to note the greater variance for both norepinephrine and serotonin in the BALB/c which might be related to some difference in the mechanism for the binding of the amine molecules. These greater variances suggest, too, that if these amines are important for some behavioral parameter, they, too, should show greater variance within the BALB/c strain whereas they should not within the C57 bl/10 strain.

The factors responsible for the differences between the findings of Caspari (*2*) and those of our report are not clear but, as noted earlier, may be due to his use of total, pooled brain specimens with the expression of serotonin values in micrograms per gram (*7*).

J. W. Maas

Adult Psychiatry Branch, National Institutes of Health, Bethesda 14, Maryland

References and Notes

1. J. P. Scott, *J. Heredity* **33**, 11 (1942); B. Ginsburg and W. C. Allee, *Physiol. Zool.* **15**, 485 (1942); G. Lindzey, *J. Comp. Physiol. Psychol.* **44**, 389 (1951); W. R. Thompson, *Can. J. Psychol.* **7**, 145 (1953); D. D. Thiessen, *Psychol. Rec.* **11**, 299 (1961).
2. E. Caspari, paper presented at Am. Psychol. Assoc. annual convention (1960).
3. B. B. Brodie, K. F. Finger, F. B. Orlans, G. P. Quinn, F. Sulser, *J. Pharmacol. Exptl. Therap.* **129**, 250 (1960).
4. R. Kuntzman, P. A. Shore, D. Bogdanski, B. B. Brodie, *J. Neurochem.* **2**, 226 (1961).
5. M. H. Aprison and C. B. Ferster, *ibid.* **6**, 350 (1961).
6. H. Weil-Malherbe, H. S. Posner, G. R. Bowles, *J. Pharmacol. Exptl. Therap.* **132**, 278 (1961).
7. Assistance with the statistical handling of the data by Dr. Donald Morrison, the technical assistance of Mr. H. Landis, and the suggestions of Dr. R. Kuntzman and Mrs. C. Hirsch as to biochemical methodology are appreciatively acknowledged.

1 June 1962