PRESENCE OF SPECIES SPECIFIC ANTIGENS IN TISSUES

Wilmer J. Miller
University of California
Davis, California

Preliminary studies were begun in 1950 at the University of Wisconsin to detect the presence in body tissues of antigens named A, B, C, and E present on the erythrocytes of Columba guinea, the triangular spotted pigeon, in contrast to C. livia, the domestic pigeon (Irwin, Cole and Gordon, 1936). These studies were never completed. However, the results obtained are thought to be of sufficient interest to warrant brief presentation.

Three birds, hybrids of guinea with livia from several backcrosses to livia, served as the main source of organs and tissues. By agglutination tests, G80J2 was determined to possess only B, a male hatched 25 Jul 49 was found to possess only C, and G177P2 had only E of guinea. These birds were killed and frozen immediately. Other similarily identified birds were used for the tests of plasma.

Simple inhibition tests were conducted with plasma from backcross pigeons each possessing a single antigen of guinea. An antiserum produced in a rabbit against red blood cells of C. picazuro and absorbed by red cells of livia was the best means of detecting of A of guinea during the period of these tests. When this anti-picazuro serum was mixed in a 1:1 ratio with (heated or non-heated plasma) from A birds for 45 minutes at room temperature, the resulting mixture still reacted to the same strength and titer (1/240) with A cells as the original antiserum. A potent antiserum 473F7 produced in a rabbit against erythrocytes of guinea, then abosrbed with erythrocytes of livia, was used in similar inhibition tests with plasma of birds possessing B, C or E. This reagent titered 1/480 (four doubling dilutions from 1/60, the basic dilution for the absorptions) with erythrocytes possessing B or E of guinea and 1/1920 (6 doubling dilutions from 1/60) with erythrocytes possessing C of guinea. After the inhibition tests the titers (indicated above) were the same as before the inhibition for the appropriate reactive cells. Indeed the plasma enhanced the degree of agglutination. Thus, it is not likely that plasma of birds whose erythrocytes possess A, B, C or E contains such specificities.

The technic of Boorman and Dodd (1943) was generally followed with thawed tissue from the frozen birds, but with one important modification--the addition of "neutral" plasma from livia as indicated below. The tissue or organ was cleaned of extraneous material (as fat), cut into small chunks and ground for a few minutes with distilled water in a Waring blendor. Lung tissue took three times as long to grind as most others, and skin and oviduct could not be so ground at all (the oviducts in the present study were cut into tiny flakes by a scissors and scapel). The tissue was washed twice in distilled water and then twice in saline. It was then mixed and incubated for 30 minutes at room temperature with plasma from livia at a ratio of 1 part tissue to 2 of plasma. Without this modification a great amount of "non-specific" absorption occurred.

Such "non-specific" absorption was demonstrated by using reagents containing reactive specificities for antigens lacking in the bird whose tissue was

being used. Such reagents lost much of their strength if the plasma addition was omitted, but little loss occurred if it was used prior to the absorption. The addition of the saline wash after the distilled water wash and the addition of the plasma incubation and wash after the saline wash, always removed considerable cellular debris regardless of the clearness of the supernate from the last wash.

After the plasma incubation the tissue was centrifuged, the supernatent removed and the specific antiserum (473F7), previously absorbed by red cells from many livia, added to the packed tissue at a 1:1 ratio for 15 minutes at room temperature and mixed. The mixture was then centrifuged and the supernatant removed and titered against B, C, E and livia cells, just as from any absorption. The effect on the titer is presented in the accompanying Table I. For example, absorption of 473F7 by kidney tissue of a bird possessing C reduced the titer of C specificities by 5 doubling dilutions (e.g. from 1/1920 to 1/60). Since dilutions of the reagent lower (more concentrated) than 1/60 were not made at that time, the total reduction or inhibition ability of the tissue is unknown for 7 of the combinations in which no titer was left after absorption by the tissue.

Non-specific absorption was still evident to 1 or 2 doubling dilutions even after the plasma treatment, as determined by the reduction in titer for antigens lacking in the bird. For example, perhaps tissue from a bird possessing C reduced the titer of the 473F7 reagent for cells possessing E from 4 to 3 doubling dilutions (from 1/480 to 1/240). Thus a reduction in titer of 1 or 2 doubling dilutions, as exhibited by the absorbtion of 473F7 with liver tissue from a C bird and testing the supernatant with C cells may not be significant.

Lung and kidney tissue reduced specific titers significantly in all the absorption tests. Liver tissue did so for B and E titers but not for C reagent titers. Muscle, oviduct and brain tissue did not affect the titers significantly.

Perhaps the most serious criticism of the study, other than its incomplete nature including lack of replication, is the lack of knowledge concerning the erythrocytic debris left in the tissue after the various washes. Certainly future efforts in this direction could improve the procedure by using fresh killed birds (no freezing to break the red cells) and perfusing and replacing as much blood as possible by saline. However, the lack of significant absorption or inhibition by muscle or by liver of birds possessing C would seem to indicate that the washing procedures were quite effective in removing red cell debris.

TABLE I

Titer in number of doubling dilutions from 1/60

Bird	type	473F7 absorbed by livia	Titer with red cells of the same type as those of the birds from which the tissue was taken					
			lung	kidney	liver	muscle	oviduct	brain
♀ G80J2	B	4	0	0	0	2	3	3
♂ 25 Jul 49	C	6	0	1	4	4	-	6
♀ G177P2	E	4	0	0	0	3	4	4

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