Although it is known that polyunsaturated C_{20} and C_{22} fatty acids are present in quantity in most fish oils, dogfish-liver oil was found to contain substantial amounts of C_{20} and C_{22} monoenoic fatty acids as well. The following isomers were found in the oil: *cis*-9-hexadecenoic acid (6.1%), *cis*-9-octadecenoic acid (24.7%), *cis*-11-octadecenoic acid (9.8%), *cis*-9-eicosenoic acid (4.0%), *cis*-11-eicosenoic acid (5.1%), *cis*-11-docosenoic acid (6.7%), and *cis*-13-docosenoic acid (1.8%). It is suggested that most of the monoenoic fatty acids of dogfish-liver oil may be formed by addition of acetate units to oleic acid and other fatty acids having a Δ9 double bond.

1. Smith, C. R., Jr., Bagby, M. O., Miwa, T. K., Lohmar, R. L., Wolff, I. A., *J. Org. Chem.*, 1960, v25, 1770.

2. Bergmann, W., Creighton, S. M., Stokes, W. M., *ibid.*, 1956, v21, 721.

3. James, A. T., Webb, J. P. W., Kellock, T. D., *Biochem. J.*, 1961, v78, 333.

4. Malins, D. C., *Chem. and Ind.*, 1960, 1359.

5. Butler, C., *Fishery Leaflet* 233, U. S. Fish and Wildlife Service, 1955, 12.

6. Stoffel, W., Ahrens, E. H., Jr., *J. Lipid Research*, 1960, v1, 139, and references contained therein.

7. Vandenheuvel, F. A., Vatcher, D. R., *Anal. Chem.*, 1956, v28, 838.

8. Craig, B. M., Murty, N. L., *J. Am. Oil Chemists' Soc.*, 1959, v36, 549.

9. Haverkamp-Begemann, P., Keppler, S. G., Boekenoogen, H. A., *Rec. Trav. Chim.*, 1950, v69, 439.

10. Fulco, A. J., Mead, J. F., *J. Biol. Chem.*, 1960, v235, 3379.

11. Schlenk, H., Gellerman, J. L., *Anal. Chem.*, 1960, v32, 1412.

12. Markley, K. S., *Fatty Acids*, 2nd Ed., Part 1, Interscience Publishers, Inc., New York, 1960.

13. Hopkins, C. Y., Chisholm, M. J., *Can. J. Chem.*, 1954, v32, 1033.

14. Lovern, J. A., *Biochem. J.*, 1937, v31. 755.

15. ———, The Chemistry and Metabolism of Fats in Fish, *The Biochemistry of Fish*, Edited by R. T. Williams, Biochem. Soc. Symposia No. 6, Cambridge Univ. Press, Cambridge, England, 1951.

Received July 5, 1961. P.S.E.B.M., 1961, v108.

Passive Cutaneous Anaphylaxis in Mice and Chickens. (26869)

FRANCO CELADA* AND ALICIA RAMOS† (Introduced by T. Makinodan)
Biology Division, Oak Ridge National Laboratory,‡ Oak Ridge, Tenn.

That the homograft reaction has many properties in common with delayed-type hypersensitivity is an established fact. Billingham *et al.*(1) demonstrated the passive transfer of the reactivity against skin homografts in guinea pigs is by cells and not by serum. The radiosensitivity of the homograft reaction(2,3) is of the same order of magnitude as that of the delayed hypersensitivity reaction(4,5), and much lower than that of soluble antibody formation(6). Nevertheless, antibodies directed against cell antigens often have been found in the serum of animals presensitized with homologous leukocytes(7) or lymphoidal cells(8,9), or bearing skin homografts(10,11). These circulating antibodies were detected by determining their cytotoxic activity *in vitro*(8,11,12) or by their ability to suppress antibody-forming activity of donor-type lymphoidal cells *in vivo* (9,13). Thus, it is apparent that the mechanism of rejection of homologous tissues is a complex one, and the relative role played by different factors is not well known; in order to understand this mechanism better, it seems necessary to use classical immunological methods along with *in vivo* assays for homograft reactions.

Recently, in other laboratories as well as in ours, transplantation antigens have been obtained in soluble form(14-16). Using these antigens, one can take a serological approach

* Portion of this work was performed during a Nat. Inst. Health Postdoctoral Fellowship.

† Visiting investigator from Inst. de Biologia, Univ. de Chile, Santiago, Chile.

‡ Operated by Union Carbide Corp. for U. S. Atomic Energy Commission.

both to the question of the relative specificities of cells and serum and to identification of the antigens. We have used the passive cutaneous anaphylaxis (PCA) reaction with a well-known antigen-antibody system in the mouse as a preliminary step toward this approach. Our primary interest is in transplantation problems in mice; we included also some experiments of PCA in chickens because data are lacking on the subject, and because a great part of the embryological studies and many investigations on development of the immune machinery and on tolerance(17) have been conducted in those animals. The present study was limited to the following objectives: (a) to test the findings of Munoz and Anacker(18) on sensitivity of PCA in the mouse; (b) to determine whether the phylogenetic relationship between source of antibodies and the test animal influences the sensitivity of the test in mice (isologous *vs* homologous *vs* closely related heterologous *vs* distantly related heterologous), a trend indicated in the guinea pig by Ovary(19), (c) to develop a satisfactory reverse passive cutaneous anaphylaxis (RPCA) technic in the mouse, and (d) to develop a satisfactory PCA technic in the chicken.

Materials and methods. 1. *Animals.* Twelve to 16-weeks-old C31F_1/Cum (C3H/Anf Cum ♀ × 101/Cum ♂) mice (H-2^{kk}), to be henceforth referred to as (C3H × 101)F_1, and LAF_1/J (C57L/J ♀ × A/HeJ ♂) mice (H-2^{ba}), to be henceforth referred to as LAF_1, were used. Their approximate weight was 25 g. Three-weeks-old white leghorn chicks weighing ~ 150 g were used.

2. *Antigens.* Crystalline bovine serum albumin (BSA) (Pentex Co.), was used in most experiments. Solutions of BSA were prepared the same day of experiment in 0.15 M NaCl in the case of RPCA, and in 0.15 M NaCl containing 0.5% Evans Blue dye in the case of PCA. Rat whole serum was used in one set of experiments.

3. *Antisera.* Anti-BSA sera were obtained from rabbits, chickens, rats (Sprague-Dawley), and mice (C3H × 101)F_1, and titrated quantitatively for their antibody nitrogen (AbN) content(20). The μg AbN/ml for the various sera was as follows: rabbit anti-BSA, 340; chicken anti-BSA, 588; rat anti-BSA, 134; mouse anti-BSA, 168. Chicken and mouse antisera against rat serum were produced respectively by repeated intravenous injection of whole rat serum in full-grown chicken, and by repeated intraperitoneal injection of rat serum in Freund's adjuvant in (C3H × 101)F_1 mice. Because of the complexity of the antigenic material, it was not possible to determine the AbN content of these antisera; their relative "strength" was estimated by the interfacial method (ring test) using the usual 4-fold dilution method. Then the titers of these antisera were adjusted to 4^{-5} by appropriate dilution with 0.15 M NaCl.

Two-fold serial dilutions of the antisera were always made on the same day of experiment. The diluent was 0.15 M NaCl, except in the RPCA experiments. In this case non-reactive Tyrode's solution was used as a diluent after we observed that in some cases 0.15 M NaCl controls showed weak non-specific reactions.

4. *PCA in mice.* PCA was induced according to the technic of Ovary(21) and Munoz and Anacker(18) with slight modifications. A very mild anesthesia was induced by leaving the animals in an atmosphere saturated with ether for 40 seconds. During the anesthesia the back was shaved gently with an electric clipper, a longitudinal line was drawn along the center of the back with a felt pen, and 0.05 ml of antiserum and 0.15 M NaCl control, or of 2 different dilutions of the antiserum were injected intradermally right and left of the central line using a 27-gauge needle. By the end of this operation the animals were almost completely awake. After 3 hours, 1.25 mg of BSA (0.025 mg/g body weight) in 0.25 ml of saline containing 0.5% Evans Blue was injected into the lateral tail vein. When rat serum was used as an antigen, the amount of protein injected was 2.5 mg. The mice were decapitated 30 minutes later and the inner surface of the skin was examined for dye accumulation. The reactions were scored from 1+ to 4+ according to their diameter, as reported by Ovary(21). The 2 negative controls were (a) 0.15 M NaCl instead of the antiserum,

and (b) a challenging saline injection containing dye without the specific antigen.

5. *PCA in chickens.* This test was conducted with the same time sequence as that in the mice. No anesthesia was necessary; 0.1 ml antiserum or 0.15 M NaCl control was injected intradermally in the lateral thoracic region. We found that upon delivery the fluid easily diffuses into the hypodermal tissues; to avoid this diffusion, the direction of the needle was reversed after penetrating the skin, and the antisera was injected in the superficial layers. The immediate appearance of a satisfactory injection was a whitish wheal, which disappeared in 10 minutes. The antigen was injected by the wing vein (10 mg in 2 ml of the standard BSA-dye solution).

6. *RPCA in mice.* This test was performed using the 2 technics of Ovary(19). According to the first technic, BSA was injected intravenously (2.5 mg/mouse or 0.1 mg/g of bodyweight). Twenty-four hours later, 0.05 ml of an appropriate dilution of the antiserum was injected intradermally as in the direct PCA, and 10 minutes later 0.25 ml of the standard dye solution was injected intravenously. The animals were killed 30 minutes later and the reactions evaluated. Our modification of this technic was an increased delay between injection of antiserum and the dye; we found that when the dye was injected simultaneously with the antiserum, a weak false positive reaction always appeared in the controls; the interval we used was the shortest that allowed us to have completely negative controls (approximately 10 minutes).

According to the second technic, the mice were inoculated with 0.002 mg antigen (BSA) intradermally, then injected intravenously with 0.25 ml of antibody dilutions plus 0.25 ml Evans Blue at the standard concentration. The reactions were read 30 minutes later.

Results and discussion. Experiment 1. PCA in mice with isologous vs *homologous antiserum.* The test was performed in (C3H $\times$ 101)F_1 and LAF_1 mice, and anti-BSA serum was obtained in (C3H $\times$ 101)F_1 mice. In both recipients the minimum amount of anti-BSA required to give a positive reaction was 0.007 μg AbN. Therefore, in this combination of strains there is no difference of sensitivity of PCA between isologous and homologous systems. The sensitivity of the reaction in mice confirms the findings of Munoz and Anacker(18) and under these conditions is comparable with that in guinea pigs(21).

Experiment 2. PCA in mice with antisera from different sources. The PCA test was performed in (C3H $\times$ 101)F_1 mice with mouse [(C3H $\times$ 101)F_1], rat, rabbit and chicken anti-BSA sera. The results (Table I) clearly demonstrate that the sensitivity of the test in mice with the 4 antisera used is dependent upon the phylogenetic relationship between source of antiserum and recipient; *i.e.,* mouse > rat > rabbit > chicken antiserum. Note that chicken antiserum failed to elicit a positive reaction even with

TABLE I. PCA and RPCA in Mice* (BSA-Anti-BSA Reaction).†

Dose of antibody (μg AbN per site) intradermally	Source of antiserum							
	Mouse		Rat		Rabbit		Chicken	
	PCA	RPCA	PCA	RPCA	PCA	RPCA	PCA	RPCA
2.0	++++	++++	++++	++++	++	+++	—	—
1.0	++++	++++	+++	++	++	++	—	—
.5	++++	+++	+++	++	+	+	—	—
.25	++++	++	++	+	+	—	—	—
.125	+++	++	+	+	—	—	—	—
.062	+++	+	+	—	—	—	—	—
.031	++	—	—	—	—	—	—	—
.015	+	—	—	—	—	—	—	—
.007	+	—	—	—	—	—	—	—
.003	—	—	—	—	—	—	—	—

* According to Ovary's "first technic"(19).
† Antigen dose per animal was 1.25 mg for PCA and 2.5 mg for RPCA.

2 μg of AbN. The results obtained with rabbit antiserum agree with those reported previously by Ovary(21) under comparable conditions.

Experiment 3. RPCA in mice with antisera from different sources. In the guinea pig, horse antisera cannot elicit a positive PCA reaction, but they can trigger a positive RPCA reaction, provided the test antigen is a globulin from a source closely related to the recipient (*e.g.*, rabbit gamma globulin) (19). Reasoning from this type of observation, Ovary deduced that the RPCA reaction is dependent upon the binding capacity of the antigen to the recipient tissues. If this is true, RPCA could be used to screen antigen-recipient affinities and may have an application in the study of transplantation antigens and of γ-globulin allotypes. It was of greater interest for us to know if and to what extent in the mouse RPCA might be dependent on "antibody-binding." Therefore, we determined RPCA according to Ovary's first technic with the same antisera that we had used in the PCA tests. We expected that the BSA antigen, being distantly related to the mouse, would not "bind" the recipient's tissues. This was confirmed by the negative reaction observed with chicken anti-BSA serum (Table I, last column). RPCA reactivity patterns were similar to those observed with PCA, except for a slight decrease in sensitivity (Table I). The delay between antiserum and dye injections could have been sufficient to permit "binding" of the injected antibodies to the tissues with a consequent reaction mechanism no different from that of the PCA. An experiment was performed to test this possibility using Ovary's "second technic" of RPCA(19), in which no delay between antiserum and dye exists. Again the same reactivity patterns were obtained (Table II). Thus, these results suggest that the antibodies can bind the recipient tissues during the reaction itself when they are of homologous or closely related heterologous origin. It can be concluded that, in order to have a reaction solely dependent on antigen-recipient relationship, only distantly related heterologous antisera should be used to perform RPCA in the mouse. To investigate the effect of an antigen more closely related to the mouse than BSA, whole rat serum was used as an "antigen," which we assumed would bind to some extent the recipient tissues, especially because of its globulin content. This reaction was also dependent upon the source of antiserum (Table III). The slightly positive reactions obtained with relatively high concentration of chicken antiserum in experimental animals are not significant since comparable results were obtained with the controls (column 3). It is likely that these false-positive reactions reflect a cross-reaction between chicken antibodies directed against rat serum proteins and mouse tissue antigens, for we have observed in Ouchterlony plates precipitin band formation between chicken anti-rat serum and mouse serum proteins.

Experiment 4. PCA in chickens with homologous and heterologous antisera. PCA can be demonstrated in chickens, although its sensitivity is considerably lower than that in

TABLE II. RPCA* in Mice (BSA-Anti-BSA Reaction).†

Dose of antibody (μg AbN) intrav.	Source of antiserum	
	Mouse	Chicken
84	++++	—
21	++	—
5	++	—
1.25	+	—
.31	—	—
.07	—	—

* According to Ovary's "second technic"(19).
† Dose of antigen inj. per site: 2 μg.

TABLE III. RPCA* in Mice with Anti-Rat Sera.†

Antiserum dilution	Source of antiserum			
	Chicken	Mouse	Chicken	Mouse
	(Experimental)		(Control)‡	
1	++	++++	++	—
1:2	+	+++	+	—
1:4	±	+++	±	—
1:8	—	+++	—	—
1:16	—	+++	—	—
1:32	—	+++	—	—
1:64	—	++	—	—
1:128	—	+	—	—
1:256	—	—	—	—

* According to Ovary's "first technic"(19).
† Chicken and mouse antisera had the same interfacial titer (4^{-5}).
‡ No antigen.

TABLE IV. PCA in Chickens (BSA, Anti-BSA Reaction).*

Dose of antibody (μg AbN/site)	Source of antiserum	
	Chicken	Mouse
10	++++	++
5	+++	++
2.5	+++	±
1.25	++	—
.62	++	—
.31	+	—
.15	—	—

* Antigen dose/animal = 50 mg (0.3 mg/g of body wt).

the mammals (Table IV). Again we found that in chickens sensitivity is higher with homologous than with distantly related heterologous antiserum against the same antigen. Although no reaction was elicited by chicken antiserum in the mouse (Table I, column 8), mouse antiserum was capable of inducing a positive though weak PCA reaction in chickens.

Summary and conclusions. The sensitivity of the PCA test with different anti-BSA sera was tested in mice. Sensitivity is greatest with isologous or homologous antisera; *i.e.*, as little as 7×10^{-3} μg AbN produced a positive reaction. In contrast, 62 and 250×10^{-3} μg AbN were necessary when rat and rabbit antisera were used, respectively; when chicken anti-BSA was used, the test was negative with as high as 2 μg AbN. The sensitivity of the PCA reaction in mice with isologous and homologous antiserum suggests that it could be a useful tool in screening for circulating antibodies against transplantation antigens. PCA in chickens is described for the first time. Although the sensitivity of this reaction relative to that in mammals is low, it can be elicited with a distantly related antiserum, such as mouse anti-BSA. RPCA in mice was found to depend on the antiserum-recipient relationship when either BSA or rat whole serum was used as antigen.

It is a pleasure to acknowledge the advice and helpful criticism of Dr. T. Makinodan of this laboratory and of Dr. Z. Ovary, New York University.

1. Billingham, R. E., Brent, L., Medawar, P. B., *Proc. Roy. Soc. B.*, 1954, v58, 143.

2. Bain, G. O., *Canad. J. Biochem. Physiol.*, 1960, v38, 621.

3. Celada, F., Makinodan, T., *J. Immunol.*, in press.

4. Salvin, S. B., Smith, R. F., *J. Exp. Med.*, 1959, v109, 325.

5. Uhr, J. W., Scharff, M., *ibid.*, 1960, v112, 65.

6. Celada, F., Carter, R. R., *Fed. Proc.*, 1961, v20, 33.

7. Harris, T. N., Harris, S., Farber, M. B., *ibid.*, 1961, v20, 40.

8. Basch, R., Stetson, C. A., Jr., *ibid.*, 1961, v20, 39.

9. Celada, F., unpublished data.

10. Amos, D. B., Gorer, P. A., Mikulska, B. M., Billingham, R. E., Sparrow, E. M., *Brit. J. Exp. Path.*, 1954, v35, 203.

11. Terasaki, P. I., Cannon, J. A., Longmire, W. P., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 280.

12. Gorer, P. A., Mikulska, Z. B., *Cancer Res.*, 1954, v14, 651.

13. Harris, T. N., Harris, S., Farber, M. B., *J. Exp. Med.*, 1958, v108, 21.

14. Castermans, A., Oth, A., *Nature*, 1959, v184, 1224.

15. Manson, L. A., Foschi, G. V., Duplan, J. F., *Fed. Proc.*, 1961, v20, 37.

16. Celada, F., Anderson, N. G., Makinodan, T., *Radiation Res.*, 1961, v14, 455.

17. Murphy, J. B., *J. Exp. Med.*, 1914, v19, 181.

18. Munoz, J., Anacker, R. L., *J. Immunol.*, 1959, v83, 502.

19. Ovary, Z., *Immunology*, 1960, v3, 19.

20. Lanni, F., Dillon, N. L., Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 427.

21. Ovary, Z., *J. Immunol.*, 1958, v81, 355.

Received June 22, 1961. P.S.E.B.M., 1961, v108.