THE PERSISTENCE OF BOVINE $\gamma$-GLOBULIN INJECTED AS AN ANTIGEN INTO RABBITS

A COMPARISON WITH ITS PREVIOUSLY STUDIED PERSISTENCE IN MICE

BY PHILIP D. McMASTER, M.D., HEINZ KRUSE, ERNEST STURM, AND JOSHUA L. EDWARDS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research)

PLATE 45

(Received for publication, June 10, 1954)

Previous studies from this laboratory (1, 2) have indicated that certain foreign protein antigens, among them bovine $\gamma$-globulin, injected into the blood stream of mice, may persist in the blood for about 8 weeks and in certain tissues for nearly 14 weeks. It is to be recalled that the detection of minute traces of antigen, persisting in the blood or tissues of these mice, was carried out at various intervals after the injection by transferring blood or ground tissues from these animals, as “donors,” to the peritoneal cavities of normal, “recipient” mice which were challenged 2 days later by an intravenous injection of a strong, anti-bovine $\gamma$-globulin rabbit serum. If antigen was present in the transferred materials and had been absorbed by the recipients in sufficient amounts to sensitize them, certain specific vascular responses of reversed passive anaphylaxis appeared in the smaller, and sometimes too in the larger, blood vessels of the ears when the latter were examined under the microscope. These reactions have been termed “ear vascular responses, EVR.” In mice receiving very minute amounts of antigen the specific EVR, though weak, can be distinguished from other, non-specific vascular responses.

The persistence of antigen, as indicated by this sensitive test (1), the “mouse transfer test,” appears to be longer than that found by many workers who have investigated the persistence of antigens by other means and in other species. The work of some authors (3–24), however, has pointed to a long persistence of certain antigens in the rabbit, guinea pig, and man. Hence the question arose whether the long persistence of injected antigen in mice (1) is a species’ peculiarity. Mice do not form precipitins well, and in consequence injected foreign antigens might remain intact for relatively longer periods than in other animals if not subjected to the influence of high concentrations of circulating antibody.

To investigate this possibility the persistence of an antigen was studied in animals which form antibody relatively well, employing the same general plan of attack as in the previous work with mice (1). That is to say, bovine
BOVINE \(\gamma\)-GLOBULIN INJECTED AS ANTIGEN

\(\gamma\)-globulin\(^1\) was injected once only into the blood stream of rabbits, as donors, in the same amount per gram of body weight (1.0 mg. per 10 gm.) as that previously given to donor mice, and at various intervals thereafter serum or ground liver tissue, taken from these donor rabbits, was transferred to the peritoneal cavities of mice. These recipient mice were tested for the development of sensitivity to the antigen that might be persisting in the transferred materials. As will be detailed, utilization of the mouse transfer test showed that bovine \(\gamma\)-globulin, as an antigen, persisted in the liver of rabbits, following its injection into the blood, for at least 2 months, and in the blood itself for more than a month, even at times for 6 weeks.

**The Need for Increasing the Sensitivity of the EVR Detection Test for Transferred Antigen.**

The problem obviously called for the detection of small, dwindling traces of injected antigen in animals believed capable of destroying it rapidly. Consequently all mice employed for EVR tests should possess a high degree of anaphylactic sensitivity. The mice used for the earlier work (1), an outbred strain (Rockefeller Institute mice), possessed an extreme degree of anaphylactic sensitivity, yielding positive EVR tests when sensitized, intraperitoneally, with as little as 0.5 to 0.1 \(\mu\)g. of bovine \(\gamma\)-globulin and challenged intravenously with strong antiserum. By the time the present work was begun the sensitivity of the mice of the same strain had changed, for some reason, to such a degree that they required sensitizing injections of as much as 20 to 30 \(\mu\)g. to yield ear reactions. As a result the animals became useless for the detection of minute traces of antigen. To be sure, fluctuations in sensitivity occurred but they were unpredictable. Because of this finding much time was spent investigating the sensitivity of various inbred strains of mice to reversed passive anaphylaxis. After many trials Bar Harbor C mice were found to be almost as responsive as the mice originally used, but after a few months these animals, too, became far less sensitive than they had been and no longer served well for use in the detection tests. This phenomenon, occurring in an inbred strain of mice, indicated that further search for naturally sensitive strains would lead as before to only temporary success. Clearly the project would have to be abandoned unless means could be found for artificially increasing the anaphylactic responsiveness of the test mice.

**Procedures Developed to Improve the Delicacy and the Usefulness of the Mouse Transfer Detection Test**

Before attempting the detection of the minute traces of antigen that one would expect to find persisting in materials transferred from rabbits injected with it, various procedures were developed to improve the delicacy and usefulness of the mouse transfer detection test. These must first be outlined in some detail, although the techniques employed for these studies have already been fully described (1) and the methods used for the detection of antigen persisting in rabbits are detailed in the latter part of this paper.

**Adrenalectomy and Increased Anaphylactic Sensitivity.**

Recently, adrenalectomized mice have been reported by others (25–27) to show much more severe active and passive anaphylactic shock than intact mice when both sorts of animals receive huge

---

\(^1\) Bovine \(\gamma\)-globulin, fraction II, Armour and Co.
sensitizing and shocking doses of antigen. It seemed likely from these reports that adrenalectomy could render mice more susceptible also to reversed passive anaphylactic shock. Scouting experiments with several different strains of mice not only showed this to be the case but indicated that the animals became so sensitive that the EVR could again be used to detect the presence of minute amounts of bovine γ-globulin persisting in animals previously injected with that antigen.

Since it is well known that adrenalectomized mice show a decreased resistance to both physical and chemical insults, it became necessary, before sensitized, adrenalectomized mice could be used with confidence for routine EVR tests, to determine what sort of vascular reactions might appear in the ears of unsensitized, adrenalectomized, control mice following intravenous injections of such sera or protein solutions as would be employed in the routine detection tests.

Non-Specific Vascular Reactions in the Ears of Adrenalectomized Mice During and Following Intravenous Injections of Serum.—A preceding paper (1) has fully described certain non-specific vascular reactions that occur in the ears of anesthetized, intact mice during and after injections of various sera into the tail veins. Attention was called especially to a “non-specific injection reaction” and to pain reactions, both of which, since they were characterized by brief constrictions of the smaller blood vessels of the ears, had to be differentiated from the true, specific EVR. These non-specific reactions appeared also in the present work in the ears of adrenalectomized mice, and need no further discussion. Suffice it to say here that, as in the preceding work, all recipient mice showing pain reactions during challenge, were discarded, and the non-specific injection reactions were avoided as much as possible by injecting only 0.08 to 0.1 ml. of the challenging serum per 30 gm. of body weight, and by allowing at least 40 seconds for the injection. In the earlier work, in which only intact mice were used, larger or speedier injections could often be given without bringing on the “injection reactions.” By contrast, in the present work when challenging injections of serum larger than the limit just mentioned were given to adrenalectomized mice, however slowly—or, even if smaller injections were given too rapidly—there occurred, 3 to 6 minutes after the injection, an additional, non-specific vascular reaction hitherto not observed by us. It consisted of a progressive slowing of blood flow, dilatation of the vessels, intense congestive hyperemia, and, perhaps, stasis and death. Since slowing of blood flow is one of the signs of the specific EVR, it was found essential to avoid this non-specific reaction of adrenalectomized animals by never exceeding the limits described above for the challenging injections.

The Effect of Anesthesia upon the Adrenalectomized Test Mice.—All observations of the ear vascular reactions (EVR) must be made upon the motionless ears of anesthetized mice. In the preceding work upon intact animals pentobarbital, given as described (1), served well in spite of the fact that previous work from this laboratory (28) had shown that it lowered the blood pressure and sometimes slightly reduced the flow of blood in the ears. In the present work blood flow in the ears of unsensitized, adrenalectomized, control mice frequently appeared to be slower than in normal mice, and the anesthetic, given as previously described (1), when followed by an intravenous injection of antiserum, led to a pronounced further slowing of flow. Since, as just mentioned above, a reduced blood flow in the ears of a sensitized animal following an injection of specific antiserum is one of the signs of specific EVR, its occurrence in adrenalectomized, control mice would prevent the use of such animals for the detection test. The difficulty was avoided by injecting intraperitoneally only 0.6 mg. of pentobarbital per 10 gm. of body weight. This dose, which is about 80 per cent of that previously used (1), was just enough to keep the mice quiet while the EVR occurred. Under these circumstances normal, unsensitized, bilaterally adrenalectomized mice, injected with the
same specific antisera used with sensitized ones, showed only an improvement in the circulation of the ears; while by contrast, the sensitized ones showed specific EVR.

The Optimal Time Interval between Adrenalectomy and Sensitization.—Batches of mice, 10 in each, were bilaterally adrenalectomized, under light ether anesthesia, and sensitized by intraperitoneal injections of bovine γ-globulin ranging in amount from 500 μg. to as little as 1 μg. The mice of the first batch were sensitized on the day of the operation, but the other groups were sensitized respectively on the 1st, 2nd, 4th, 5th, 6th, 8th, 10th, and 12th days after adrenalectomy. Forty-eight hours after sensitization the animals of each group were challenged by intravenous injections of specific rabbit anti-bovine γ-globulin serum. Both the sensitized and the control mice challenged soon after the operation—on the 2nd or 3rd day thereafter, or later, on the 8th to 12th days—seemed to stand the challenge poorly, and even some of the controls showed circulatory failure. By contrast, when the animals were sensitized 4 to 5 days after adrenalectomy and challenged on the 6th to 7th days after the operation, the adrenalectomized controls showed only visible improvement of the circulation in their ears. All survived and exhibited neither EVR nor shock, whereas the severity of shock among the sensitized mice—as shown not only by EVR but by prolonged prostration and death of many of the animals sensitized with as little as 5 μg. or 1 μg. of antigen—was far greater than that to be expected in similarly sensitized, but intact, mice.

The high death rate and the prolonged prostration of many of these lightly sensitized mice—and the absence of these phenomena in the control mice which showed only betterment of their general condition—constituted an improvement upon the earlier detection test, which depended entirely upon the subjective decision of the observer of the EVR.

The Utilization of Adrenalectomized Mice for the Detection of Antigen in Transferred Tissue or Blood.—The preliminary tests outlined so far showed only the best time interval at which to sensitize adrenalectomized mice given a simple solution of the antigen, bovine γ-globulin. It remained to determine whether or not adrenalectomized mice could withstand the intraperitoneal transfers of serum or liver suspension which would be required if they were to serve as recipient animals for the routine antigen detection tests.

To test this point another preliminary experiment was made like that described above except for the fact that, following bilateral adrenalectomy, each recipient mouse was injected intraperitoneally with a saline suspension of 0.5 gm. of ground mouse liver in a total volume of 0.75 to 0.8 ml. The technique of transfers like these has already been described (1). Half the recipients got liver from donor mice that had been injected intravenously 2 weeks previously with bovine γ-globulin, while the remaining half got normal mouse liver. As in the preceding test some received the transfer on the day of the operation and others at daily intervals from the 2nd to the 10th day after operation. All were challenged 48 hours after the transfer to seek for the EVR of reversed passive anaphylaxis. As in the preceding tests, transfers made 4 or 5 days after operation with challenge on the 6th or 7th day served best. Even at these intervals some of the control, adrenalectomized mice, that received normal mouse liver, showed circulatory failure or changes in blood flow in the ears often

---

2 All adrenalectomies were performed by the same operator by simple avulsion with curved forceps, through a posterior incision. All were performed under ether anesthesia. Immediately after the operation, and usually at daily intervals thereafter, the mice received subcutaneous injections of 1 ml. of 1.25 per cent NaCl in 5 per cent dextrose solution. They were given 0.9 per cent NaCl solution to drink ad lib.
enough to force many tests into the discard. As result means were found to overcome this difficulty, as will now be described.

Practicability of Unilateral Adrenalectomy.—Weiser, Golub, and Hamre (25) had already shown that unilaterally adrenalectomized mice are more sensitive to active and passive anaphylaxis than intact animals, although the increased sensitivity is not as great as that which follows the loss of both adrenals. Accordingly, mice of the strains employed in this work were deprived of 1 adrenal, sensitized with known amounts of bovine γ-globulin, and challenged with antiserum to test for their sensitivity to reversed passive anaphylaxis. They were found almost as sensitive as bilaterally adrenalectomized mice provided that sensitization and challenge were performed respectively 4 to 5 and 6 to 7 days after the operation. In further tests—like those already performed with mice deprived of both adrenals—ground tissues or sera were transferred to recipient mice lacking only one of the organs. The degree of anaphylactic sensitivity of these animals, too, seemed to be almost as great as that of bilaterally adrenalectomized mice and far greater than that of normal intact mice. Moreover, the recipient mice lacking only one adrenal withstood the transfers of tissues or blood far better than those lacking both adrenals. The controls behaved much like normal animals and stood out in sharp contrast to the animals showing positive EVR. Following these tests, unilaterally adrenalectomized mice were used for much of the work to be reported below.

Efforts to Improve the Objectivity of the Detection Test

In performing the detection test one observer, looking for positive EVR, must watch the blood vessels in the ears of the test animals under the microscope and determine whether or not specific or non-specific reactions have occurred. Many years of work employing the ears of mice for studies on injuries and burns, blood physiology, and lymph flow, have familiarized the authors with circulatory events, happening in the ear vessels, following the injection of various substances into the blood stream of mice. Workers less familiar with these events might find that the differentiation of the specific EVR from non-specific reactions requires too much preliminary study to render the detection test useful for general purposes and so, in our opinion, it well may be. Undoubtedly the usefulness of the test would be greatly enhanced if, instead of having recourse to the EVR, one could elicit, in the test animals, some of the other, unmistakable and more objective physical signs of anaphylaxis.

The physical signs of active and passive anaphylactic shock in mice receiving very large (25-27, 29-41) or moderately large (42-45) sensitizing and shocking injections have already been described. It was not known whether mice, given mere traces of bovine γ-globulin or liver suspension from donor animals previously injected with the antigen, would become sufficiently sensitive to show the gross physical signs of reversed passive anaphylaxis when challenged without anesthesia. During the work just described some of the adrenalectomized mice, to which tissues presumably
Bovine γ-globulin injected as antigen

harboring antigen had been transferred, showed such strong EVR that it seemed likely that such animals might exhibit at least some of these signs. Should they appear one would have at hand a new type of detection test for the presence of transferred antigen; not only a more objective test, capable of being watched by several workers, but also one more easily performed than the EVR test. Accordingly it seemed worth while to attempt the development of a detection test of this type.

*Gross Physical Signs of Reversed Passive Anaphylaxis Successfully Elicited in Recipient Mice*

Normal, intact mice, as well as bilaterally and unilaterally adrenalectomized animals, were injected intraperitoneally with bovine γ-globulin in amounts varying from 50 μg. to 0.5 μg. Others received 0.5 gm. of liver suspension from donor mice injected with 3 mg. of the antigen a month before. The animals operated upon received their sensitizing doses 4 to 5 days after the operation. Two days later all were challenged with specific anti-bovine γ-globulin rabbit serum, given intravenously in the absence of anesthesia. All manner of severe and mild reactions occurred. They were most severe in the mice deprived of both adrenals, but reactions almost as strong appeared in the unilaterally adrenalectomized animals. By contrast, the intact mice showed far weaker reactions and often none. Other, intact or partly and wholly adrenalectomized mice were used as controls. Some, which received the same materials as the test animals and in the same amounts, were challenged with normal rabbit serum; others were given normal rabbit liver suspension and challenged with the same antiserum that was given to the test mice. These control mice showed no signs of anaphylaxis. In these tests the mice deprived of only one adrenal served best since they were far more sensitive than intact animals, and the controls, lacking but one adrenal, behaved like normal animals.

*The Physical Signs of Reversed Passive Anaphylaxis in Unanesthetized Recipient Mice.—*As will appear below, the gross physical signs of reversed passive anaphylaxis in the unanesthetized mouse appeared to be much like those of active anaphylaxis. This was true whether or not the animals received a pure protein antigen or antigen-containing tissue suspensions. Although the signs of active and passive anaphylaxis in the mouse are well known, it is necessary briefly to describe mild reversed passive anaphylaxis in order to make clear the criteria used in the latter part of the work to determine whether or not the presence of antigen had been detected in materials transferred from donor rabbits to challenged, recipient mice.

In the test just mentioned above, each mouse, as soon as it had received its challenging, intravenous injection was placed alone in a large tray, free to move about at will. For a minute or so both the control and the test animals, disturbed by the injection, remained almost motionless. Thereafter the controls ceaselessly explored their surroundings sniffing, or rubbing the nose with the forepaws. By contrast, the sensitized mice showed some or all of the following phenomena. After the first moment, and perhaps after a little exploring, they hunched in a corner of the tray. At this time the ears often seemed blanched and the blood vessels constricted. Presently, like the controls, they rubbed their noses with their forepaws. Soon they began to do this much more frequently and to scratch the nose vigorously for longer periods. Next they began to use the hind legs to scratch the face, ears, neck, or body. There followed persistent licking or nibbling at the genitalia or anal regions and the base of the tail. These motions frequently gave way to frantic bouts of violent scratching, the animals obviously agitated and irritable. At this time the fur often became fluffed. About the 5th or 6th minute the blood vessels of the ears became more prominent. The mice
often made sudden rushes from one end of the tray to the other, or they indulged in apparently aimless running. In the next few minutes they became quiet again, making only occasional wild rushes. Frequently they showed changes in the respiratory rhythm and definite respiratory difficulty.

At this time, if a gentle stream of air was blown on them, violent startle reactions occurred, the animals rushing from a hunched position in one corner to crouch in another. A sudden noise often produced the same response. If touched they might chirp and leap in an excessive startle reaction, all 4 feet off the ground at once. None of these stimuli, if not too intense, produced any reactions in the controls except normal avoidance. Defecation and urination, more frequent on the part of the sensitized mice than in the controls, did not always occur.

The signs described so far will be referred to later in the paper as Phase I of reversed passive anaphylaxis. They appeared in differing degrees of severity, but they constituted definite evidence of a mild positive reaction. They were present, too, in severe anaphylaxis, simply occurring more rapidly and intensely than in mild reactions.

Slightly more severe reactions showed other signs that might be termed Phase II. The mice became lethargic. Often the head dropped until the nose touched the ground. Later the ears, feet, and tails became engorged or cyanotic, and the respiratory movements were more pronounced. When the animals moved they seemed weak, often leaning against the walls and dragging their hind legs. At intervals the mice came out of their lethargy and indulged in violent scratching and biting of the posterior regions.

About 20 minutes, or longer, after the challenging injection a few of the mice exhibited a more objective phenomenon, that is to say, a swelling of the lips and snout often accompanied by edema of the paws. The typical appearance of the phenomenon is shown in Figs. 1 to 3, photographs of a unilaterally adrenalectomized recipient mouse and its control. The test mouse is at the right in Figs. 1 and 3 and above the control in Fig. 2. Both mice had been sensitized by intraperitoneal injections of 0.5 ml. of serum from a rabbit injected 4 weeks previously with 1 mg. of bovine γ-globulin per 10 gm. of body weight. The test mouse was challenged 48 hours later by an injection of 0.1 ml. of strong anti-bovine γ-globulin rabbit serum. The control mouse, also unilaterally adrenalectomized, and previously injected with the same serum, was challenged with normal rabbit serum at approximately the same time as the test animal. It is to be mentioned in passing that this urticaria-like phenomenon has also been noticed in anesthetized mice examined for EVR, and therefore it cannot be attributed to scratching. Usually the mice that showed the phenomenon recovered.

Physical Signs of More Severe Reversed Passive Anaphylaxis.—Still more severe reactions, that might be termed Phase III of shock, seemed merely to intensify the signs already mentioned. Weakness increased in the hind legs until the well known “frog-leg” phenomenon developed when the mice attempted to move. Often the weakened animals lay motionless in a crouching position with their noses touching the ground, and they could be pushed about like toy mice without changing posture. Respiratory difficulty became even more pronounced. Many of the mice showing these advanced phenomena recovered.

Another objective sign appeared occasionally in mice which often recovered from shock; exophthalmos and a ground glass appearance of the cornea developed.

In more severe shock (Phase IV) the animals became prostrated and lay upon their sides. Cyanosis of the ears, noses, feet, and tails increased, and gasping respiration appeared. Animals in this phase recovered but rarely. More severe shock (Phase V) terminated in death, the animals frequently showing, just before it, convulsive movements of one or more legs, but only rarely generalized or clonic convulsions.

The Criteria for the Determination of Positive Detection Tests. Control Mice vs. Test Animals.—The control and test mice alike, after being constrained during the chal-
lenging injection seemed slightly hyperactive and apprehensive for the next minute or two. Thereafter the control mice quietly explored their surroundings, like untouched animals, whereas the test mice began to show the type of behavior just described. It is to be stressed that the controls often rubbed their noses, so that similar motions of the test animals were never considered significant unless the scratching of the nose became violent and excessive and unless this sign was accompanied by some or all of the others already mentioned. Detection tests were never called positive unless all the signs described above, as included in Phase I, were present and usually many of those included in Phase II.

In the work now to be described, all detection tests made with unanesthetized mice were compared with EVR tests carried out under anesthesia. As mentioned earlier, unilaterally adrenalectomized mice of 2 strains, Rockefeller Institute mice and Bar Harbor C mice, were employed for most of the work. In both strains reactions appeared in animals sensitized to only 1 or 2 μg., or occasionally to only 0.5 μg. Unilateral adrenalectomy seemed to render the mice about as sensitive to reversed passive anaphylaxis as the original intact animals of the Rockefeller Institute strain had been, initially, during the progress of the previous work (1) and before they had lost much of their sensitiveness, as described above. Consequently it seemed possible, with the aid of unilateral adrenalectomy, to employ mice of either of the strains tested for the detection of very small amounts of antigen in blood or tissues transferred to them. Accordingly the technique was used, as will now be described, to study the persistence of bovine γ-globulin (Armour's Fraction II) following its injection into young, adult rabbits.

A STUDY OF THE PERSISTENCE OF A FOREIGN PROTEIN ANTIGEN IN THE LIVER AND BLOOD OF INJECTED RABBITS

The primary object of this work was, as mentioned in the introductory paragraphs of the paper, to compare the persistence of bovine γ-globulin, as a protein antigen in the rabbit, a good antibody-forming animal, with the persistence of the same antigen, as already studied in the mouse (1), a poor antibody former.

Plan of the Experiments.—Twenty-three rabbits, 2700 to 3000 gm. in weight, "donors," were injected once intravenously with an aqueous 7 per cent solution of bovine γ-globulin containing 10 mg. of the protein per 100 gm. of body weight, the same amount of the antigen per gm. of weight that was given to the donor mice in the previous work. With aseptic precautions the donor rabbits were exsanguinated and various organs removed after 2, 3, 4, 6, and 8 weeks respectively. To retard the degradation of any injected antigen that might be stored in the removed tissue the organs were placed as rapidly as possible in glass dishes chilled in cracked ice. These were immediately sealed and frozen at -60°C. The blood, in paraffined tubes, was allowed to clot for 2 hours at room temperature and stored overnight at 3°C. The following day the sera, taken from the clots, were stored in 10 ml. lots and frozen at -60°C. The sera and various organs of normal rabbits were also obtained and treated in the same way.

Tests with the Sera.—The presence of both antigen and antibody was first sought by precipitin tests, using the method of Landsteiner and van der Sheer (46) and the capillary tube technique (47). Next, the presence of both antigen and antibody was sought by the mouse
transfer tests, discussed above, using young, adult, normal mice of the 2 strains already mentioned, as recipient test animals, each receiving an intraperitoneal injection of 0.5 ml. of one of the various sera. In some tests the sera were transferred to intact mice, in others to bilaterally adrenalectomized animals, but in the majority of instances to unilaterally adrenalectomized mice in which the operations had been performed 4 to 5 days before the transfer was done. Each recipient mouse was challenged approximately 48 hours after the transfer.

To detect the presence of antigen, by the EVR type of test, intravenous injections of strong rabbit anti-bovine γ-globulin sera, prepared as previously described (1) and containing 0.8 to 0.9 mg. antibody N per ml. as determined by the method of Heidelberger and Kendall (48), were carried out with all the precautions already outlined, while the animals, lightly anesthetized with pentobarbital, lay with their ears brilliantly illuminated, spread out on white porcelain plaques, under the microscope (49). During the injections, and for 20 minutes to 3 hr hour afterwards, the vessels of the animals' ears were examined at magnifications of 100 to 275 for the appearance of EVR—as also fully described in previous papers (1, 42). Unanesthetized animals, injected with the same antisera, were also observed for any apparent physical signs of reverse passive anaphylaxis, as described above.

The presence of antibody in the transferred sera was sought for by challenging some of the recipient mice with intravenous injections of solutions of the antigen, bovine γ-globulin, in various concentrations, as a 14 per cent solution, a 7 per cent solution, and as a 7 per cent solution diluted 10, 25, and 100 times respectively. As will be discussed in a subsequent paper the strongest reactions when antibody tests were positive occurred when the challenging was done with either the 14 or the 7 per cent solutions of antigen.

The Transfer of Tissues for the Detection of Antigen or Antibody Persisting Therein.—In the present work the detection of antigen persisting in the tissues of the donor rabbits was attempted only with liver. Pieces of liver tissue were cut from the frozen organs while they were still hard, and minced in chilled Petri dishes standing in finely chipped ice. The mince was immediately ground in a cooled TenBroeck grinder with a small amount of 0.9 per cent cold sodium chloride solution, and made up to the proper volume with more of the same solution, enough to include 0.5 gm. of the liver tissue in a volume of 0.7 to 0.8 ml. During these procedures the liver suspension became warmer, but, while it was still well below room temperature, enough of it was injected intraperitoneally into each recipient to transfer 0.5 gm. of liver. The animals, even the bilaterally adrenalectomized mice, tolerated the injection of the cooled material well. In this way, as also after the removal of the organs from the body, the degradation of antigen or antibody that might be present in the liver tissue was retarded, a precaution of seeming value if minute traces of persisting antigen or antibody were to be found.

Some of the recipient mice, under light anesthesia, were challenged 48 hours later with the same potent antisera, while the ears were examined for EVR. Some were challenged without anesthesia and watched for the appearance of gross signs of reversed passive anaphylaxis. Still other recipients were challenged with the various concentrations of antigen mentioned above, instead of with the antiserum, to test for the presence of antibody taken up from the transferred liver tissue.

Controls for the Tests to Detect Antigen.—In all the tests now to be described, whether carried out with sera or with liver, 3 types of controls were used. First, half of the test animals, that is to say, those which were sensitized with intraperitoneal injections of the materials suspected of containing antigen, whether partially or completely adrenalectomized or intact, were challenged intravenously, as controls, with normal rabbit serum. Next mice prepared like the test animals, either by adrenalectomy or not, as the case might be, were intraperitoneally injected with normal rabbit serum or liver tissue. For the second type of
BOVINE γ-GLOBULIN INJECTED AS ANTIGEN

Control test half of these were challenged with the antiserum used for the test animals, while, for the third type of control test, the remainder were challenged with normal rabbit serum.

Negative responses in the first type of control test indicated that the materials transferred to the test animals did not react non-specifically with rabbit serum. The second type of test, if negative, indicated that the antiserum employed did not react non-specifically with normal rabbit serum or liver tissue. The third type of test, if negative, indicated that the positive reaction seen in the test animals could not be attributed to cross reactions between normal rabbit serum or normal rabbit liver tissue and some substance present in normal rabbit serum.

Since control tests, as just outlined, were used in all the work to be described below, and only experiments which yielded negative controls have been included in this paper, nothing further will be said about controls for the antigen detection tests.

Controls for the Tests to Detect Antibody.—To control the tests for the presence of antibody transferred to the recipient mice challenged with bovine γ-globulin, other mice, prepared like them by adrenalectomy or not as the case might be, were sensitized with the same transferred materials and challenged with a non-specific protein, human serum albumin, injected in the same concentrations in which the bovine γ-globulin was used for the test animals. Other adrenalectomized or intact control mice were given intraperitoneal injections of either serum or liver tissue from normal rabbits and challenged with the same solutions of bovine γ-globulin that were used for the test mice. Since only tests in which the controls were negative have been reported, nothing further will be written about the matter.

As the experiments progressed, longer and longer periods of antigen persistence were sought. At first, when blood or liver was transferred at short intervals after injecting the donors, and the materials were relatively rich in antigen, intact rather than adrenalectomized recipients served well for its detection. In the later experiments recipients deprived of one or both adrenals were used at times and intact animals at others, depending upon the circumstances. No fixed technique was used, and consequently it will be necessary briefly to outline the tests separately to indicate the methods by which the findings from each were obtained.

FINDINGS

The Presence or Absence of Antigen and Antibody in the Sera of the Donor Rabbits after an Interval of 2 Weeks. The Results of Precipitin Tests.—Qualitative precipitin tests carried out on the serum of 2 rabbits, 2 weeks after the injection of antigen, failed to show its presence. On the other hand precipitin tests for antibody were positive at dilutions up to and including 1:5000, with the optima at 1:100 and 1:250. In Table I, which is fully described in the legend accompanying it, a minus sign stands at the head of each of columns 3 and 4 to indicate the negative precipitin reactions obtained with the sera of these 2 donor rabbits, distinguished in the table as donors A and B, respectively. The plus signs indicate the positive precipitin tests, yielded for antibody by these same sera.

Findings by the Mouse Transfer Tests.—The serum of donor A was transferred to 24 normal mice, 12 of each of the strains used. Six (3 of each strain) were challenged with strong antiserum to test for the transfer of antigen, while 6 others were challenged with antigen to
test for the transfer of antibody. The remaining animals served as controls. Although the test mice had not been adrenalectomized strong EVR appeared regardless of whether the

**TABLE I**

Table I summarizes the data indicating the presence or absence of antigen and antibody in the sera (columns 3 to 8 inclusive) or livers (columns 9 to 12 inclusive) obtained 2, 4, 6, and 8 weeks after injecting rabbits with bovine γ-globulin, as an antigen.

Columns 3 and 4 show the findings in the sera, as obtained by precipitin tests. Columns 5 to 12 inclusive represent findings in the sera (columns 5 to 8) and in the livers (columns 9 to 12) as demonstrated by “mouse transfer tests,” which have been fully described in the text. The data in the columns headed “EVR” were obtained by observing the ear vascular reactions of reversed passive anaphylaxis in anesthetized recipient mice to which, as also described in the text, either serum or liver tissue had been transferred. The data in the columns headed “Gross” were obtained by observations of the gross signs of reversed passive anaphylaxis in unanesthetized recipient mice.

Circles drawn about + or − signs indicate that the tests were carried out with intact recipient mice; whereas + or − signs without circles indicate the results of tests made with adrenalectomized recipient mice. The asterisks indicate the fact that mice in the tests so designated showed swelling of the face or paws as well as other signs of reversed passive anaphylaxis (see text).

| Donors | Precipitin tests | Mouse transfer tests |
|--------|------------------|----------------------|
|        |                  | EVR      | Gross   | EVR      | Gross   |
| 1      | 2                | 3 4      | 5 6 7 8 | 9 10 11 12 |
| Donors | antiGEN         | A B      | A B     | A B      | A B     |
| 2 weeks| antiBODY        | + +      |         |          |
| Donors | antiGEN         | C D      | C D     | C D      | C D     |
| 4 weeks| antiBODY        | + weak   | + weak  | + weak   | + weak  |
| Donors | antiGEN         | E F      | E F     | E F      | E F     |
| 6 weeks| antiBODY ft.    | − −      | − weak  | + weak   |         |
| Donors | antiGEN         | G H      | G H     | G H      | G H     |
| 8 weeks| antiBODY        | − −      | − −     | weak weak| weak weak|

animals received antiserum or antigen. In Table I, column 5, the plus signs indicate these findings. In this column, as elsewhere in the table, a circle drawn about either a plus or a minus sign indicates an experiment carried out in recipient mice with adrenals intact.
The EVR tests indicated the presence of both antigen and antibody in the serum of donor rabbit A 2 weeks after it had received antigen. The responses were so strong no tests were made with the serum of donor rabbit B. By contrast, the precipitin tests with the sera of both donors A and B, indicated the presence of antibody only, but no demonstrable antigen. In passing it can now be stated that sera obtained from rabbits a few weeks after injecting them with antigen have been shown, in this laboratory (50) as also by others (51) using methods other than the standard precipitin methods, to contain masked antigen.

Findings in Recipient Mice after Transferring Liver Tissue.—Ground liver tissue from donor rabbit A that furnished the serum for the preceding experiment was transferred to 6 normal recipient mice, and EVR tests for transferred antigen were carried out in the usual way. All were strongly positive, much stronger than those obtained by transfer of the serum from the same animal. Again, the responses were so strong that further tests with liver tissue removed from the second donor, B, were considered unnecessary. In Table I, column 9, the plus sign surrounded by a circle indicates the findings, just described, as obtained by the use of recipient mice with adrenals intact.

Clearly antigen had been transferred with the liver tissue. Since it was present in the serum of the donor rabbit, as tested for by EVR, the positive reaction might be ascribed to the presence of blood in the transferred liver. However, since the reactions were so much stronger than those yielded by direct transfer of serum, the finding indicates, but does not prove, that the liver itself contained antigen. More will be said of this below. Tests for transferred antibody were not attempted since, by precipitin tests, the serum of donor A had shown such a high content of antibody—which would of course be present in the blood contained in the transferred liver—that positive findings would be of no value.

Findings in the Sera and Livers of Donor Rabbits 4 Weeks after Injecting Antigen.—Precipitin tests on the sera from 2 rabbits (donors C and D, Table I) obtained 4 weeks after the usual single intravenous injections of antigen, yielded negative findings for antigen in both (see the minus signs in columns 3 and 4), and weakly positive tests for antibody in the serum of donor C—indicated by the plus sign over the word "weak" in column 3 in the table. The serum of donor D yielded negative precipitin tests for antibody (the minus sign in column 4).

Five normal recipient mice, with adrenals intact, injected intraperitoneally with the serum of donor C, which showed the trace of antibody, yielded no EVR reactions when challenged with antiserum, as indicated in Table I, column 5, by the minus sign surrounded by a circle. Four other recipients, challenged without anesthesia, also gave no signs of anaphylaxis, as indicated in Table I, column 7, by the same sign. These tests for transferred antigen were clearly negative. An equal number of similar recipient mice when challenged with the various solutions of antigen gave negative tests for transferred antibody by showing neither EVR nor gross anaphylaxis (as indicated in Table I, columns 5 and 7, by the minus signs surrounded by circles).

Positive tests for the presence of antigen resulted when the same serum was transferred
in the same amounts to unilaterally adrenalectomized recipient mice. Two out of 4 of these mice, challenged with antiserum under anesthesia, showed weak but positive EVR, represented in Table I, column 5, by the plus sign with the word "weak" written below it. An equal number of the recipients, challenged without anesthesia, all showed weak but definite gross signs of anaphylaxis, as indicated in Table I, column 7, by the plus sign above the word "weak."

When anesthetized and unanesthetized recipients of the same sort were challenged with antigen to test for the presence of antibody in the serum of donor C, weakly positive EVR appeared in half of the anesthetized mice, and so too, half of the unanesthetized animals gave definite objective signs of anaphylaxis like those designated earlier in the paper as Phase I. These findings appear in Table I, in columns 5 and 7, as plus signs with the word "weak" written below them.

The tests indicated the presence of very small amounts of both antigen and antibody in the serum tested.

Next, the serum of the other donor rabbit, D, injected with antigen 4 weeks previously, was tested for the persistence of antigen and antibody by transferring it to intact and to unilaterally adrenalectomized recipient mice. As in the preceding experiment, half the animals were tested under anesthesia for EVR while the other half were challenged without anesthesia to look for gross signs of anaphylaxis. In this test, controls with and without unilateral adrenalectomy were used. Tests for transferred antibody, done only with unilaterally adrenalectomized recipients, were negative (see the minus signs in columns 6 and 8 in the table). The intact recipients of the donor serum showed no reactions when challenged with antiserum to test for transferred antigen (minus signs surrounded by circles in columns 6 and 8, Table I). By contrast, the unilaterally adrenalectomized recipients, challenged with antiserum, evidenced definitely positive EVR in 3 of 6 animals tested, and positive gross signs of anaphylaxis appeared in 4 of 6 instances tested (plus signs in columns 6 and 8, Table I).

It is of much interest that 2 of the 4 mice just mentioned showed not only the clear cut objective signs of reversed passive anaphylaxis described above as Phases I and II, but also pronounced swelling of the lips and tongue and edema of the feet and lower legs, described above as a sign of Phase III. The phenomenon is indicated in Table I, column 8, by the asterisk above the plus sign. Figs. 1 to 3, alluded to earlier in the paper, show one of these mice compared with its control, which received the same donor's serum intraperitoneally but was challenged with normal rabbit serum. In this instance the facial edema began about 20 minutes after the challenge, the feet swelling about 15 minutes later. The photographs were taken 46 minutes, 52 minutes, and 70 minutes, respectively, after the challenge.

By and large the tests showed that 1 month after the injection of 1 mg. of antigen per 10 gm. of body weight to 2 rabbits, antigen could be detected in both sera by the mouse transfer test. Antibody was found in only one. Serologically no signs of antigen appeared in either of the sera but there was a weak reaction for antibody in one of them. This was the same serum which yielded positive tests for antibody by the transfer test.

The Transfer of Liver 4 Weeks after Injecting Antigen into the Donor Rabbits.—Half a gram of liver tissue from the donor rabbit C, which showed antibody in its blood, was transferred
BOVINE \( \gamma \)-GLOBULIN INJECTED AS ANTIGEN

intraperitoneally to each of 12 intact test mice. Six out of 7 of them, challenged with antisemum for EVR tests, gave positive reactions, strong in 3 instances, moderate in the others, indicating the transfer of antigen persisting in the donor's liver (Table I, column 9, the plus sign surrounded by a circle). The remaining 5 test animals, when injected, under anesthesia, with antigen in various dilutions, gave no EVR, indicating negative reactions for transferred antibody (see the minus sign surrounded by a circle, column 9, Table I). No tests were done without anesthesia to seek for gross signs of anaphylaxis, indicating the transfer of either antigen or antibody.

Liver tissue from donor rabbit D which showed no antibody in its serum by precipitin tests, was transferred to 8 recipients. In contrast to the preceding experiment, each recipient was deprived of one adrenal. All were challenged 2 days later with the same antisemur that was used in the preceding test, half under anesthesia for EVR tests and half without anesthesia. All showed strong positive reactions—EVR (plus sign, column 10) or gross signs of anaphylaxis (plus sign, column 12). So strong were they, and so much stronger than in the previous tests which employed mice with intact adrenals, that 2 of the anesthetized and 2 of the unanesthetized test mice died, and 2 of the test animals examined for EVR, under anesthesia, showed edema of the face and feet. (This finding is indicated by the asterisk over the plus sign in column 10.)

Tests for the transfer of antibody in this liver tissue were negative, as carried out in 8 unilaterally adrenalectomized recipients challenged with antigen, 4 of them anesthetized and 4 unanesthetized (see the minus signs in columns 10 and 12).

Clearly the transfer tests indicated the persistence of antigen in the rabbit liver for 4 weeks. Further, the appearance of positive EVR after the transfer of liver to recipient mice with intact adrenals, contrasts with the finding of negative EVR tests in similar animals that received serum, as described above. Apparently the liver contained much more antigen than the serum.

The Findings in Similar Tests Carried Out 6 Weeks after Injecting the Donor Rabbits.—Precipitin tests, carried out with 2 sera obtained 6 weeks after injecting donor rabbits E and F, yielded no evidence of antigen in either (minus signs in columns 3 and 4), and the presence of only a very dubious trace of antibody in one of them, donor E, as indicated in column 3 of the table by the sign "ft. +" and the minus sign in column 4.

The serum of donor E, which contained the trace of antibody, transferred to 12 unilaterally adrenalectomized mice yielded 6 negative tests for EVR, in 6 anesthetized recipients, and 6 negative tests for gross signs of anaphylaxis in the remaining 6 unanesthetized animals. Clearly antigen was not demonstrable in that serum (minus signs in columns 5 and 7, Table I). Transfer tests to another group of mice deprived of one adrenal, followed by challenge with antigen, gave negative responses for antibody (columns 5 and 7, Table I). Either the dubious trace of precipitate obtained with this serum by precipitin tests (column 3, Table I) was not due to antibody, or the biological test, as applied for the detection of antibody, was not as sensitive as the serological test. The latter possibility is favored since the mouse tests for transferred antibody have not appeared to be either as sharp or clear cut as those for antigen.

Similar tests made with the serum of donor F, which showed neither antigen nor antibody by precipitin tests (column 4, Table I) were also negative for the presence of antigen as sought for by the EVR test in anesthetized recipients (column 6, Table I). By contrast in 7 tests with unanesthetized recipients, 3 showed very weakly positive gross responses (column 8, Table I) like those described above as Phase I of gross anaphylaxis. Since the controls were all negative, as were all in each of the tests considered in this paper, the weakly
positive tests must have been specific for antigen and not brought about by non-specific factors in either the transferred or challenging materials. All tests for transferred antibody in this serum were negative (columns 6 and 8, Table I).

In summary, antigen could not be demonstrated in either serum by precipitin tests, but, by the mouse transfer test it was detectable in one, in faint traces. That is to say, 6 weeks after injecting the donor rabbits with bovine γ-globulin the antigen may or may not be demonstrable in the serum.

Antibody could not be detected in these sera by the mouse transfer tests, whereas by precipitin tests it seemed to be present, in a dubious trace, in one.

Antigen Still Present in the Liver.—By contrast, transfer tests with liver tissue from donor E, which showed no antigen in its serum (columns 3, 5 and 7, Table I), to 10 recipients with intact adrenals, gave moderately strong, positive EVR reactions in 2 instances, weak, positive EVR in 4, and 4 negative results. The finding is indicated by the plus sign surrounded by a circle (column 9, Table I). The positive reactions were definitely weaker than those obtained with liver tissue transferred after only 4 weeks. It is to be noted that in this experiment, as in some of the preceding experiments with liver transfers, the tissue was given to recipient mice that had not been adrenalectomized, although the tests for antigen in the sera of this donor had been found negative even in the more sensitive test animals with one adrenal removed.

The usual tests for transferred antibody in the liver were negative when carried out in recipient mice with intact adrenals (minus sign with a circle about it, column 9). No tests were made with these intact recipients to seek for the gross signs of anaphylaxis to indicate the transfer of either antigen or antibody.

Next, liver tissue from donor rabbit F, which showed antigen in its blood, was transferred to recipient mice. In this experiment the transfer tests with liver, like those with the serum, were carried out in recipients deprived of one adrenal. Four out of 6 of these animals (see the plus sign, column 10, Table I) showed positive and much stronger EVR than those which appeared either in the unilaterally adrenalectomized recipients, to which serum had been transferred, or in the intact recipients that got liver tissue from the other donor, E. The usual EVR tests for transferred antibody were all negative (minus sign in column 10, Table I). No tests were carried out with unanesthetized recipients.

Findings 8 Weeks after Injecting the Donors.—In 2 sera, obtained 8 weeks after injecting bovine γ-globulin into donor rabbits G and H, no antigen could be found by any of the methods used, although transfers were made not only to unilaterally adrenalectomized recipients but even to some that had been deprived of both adrenals. Antibody could not be detected in either of the sera by any of the methods used (minus signs in columns 3 to 8, Table I).

Antigen Still Present in the Liver.—By contrast transfers of liver from donor G to unilaterally adrenalectomized recipients led, upon challenge with antiserum, to 4 weak, positive EVR responses out of 5 tests (plus sign in column 9, Table I). The reactions were weaker than those obtained with tissue taken 6 weeks after injecting the donor rabbits with antigen.

Among 6 other unilaterally adrenalectomized recipients of the same donor's liver,
challenged without anesthesia for the presence of transferred antigen, 4 showed positive signs of Phase I of reversed passive anaphylaxis (plus sign in column 11, Table I). Tests for antibody, transferred in the liver, were all negative (minus signs in columns 9 and 11, Table I).

These tests were repeated with liver tissue taken from donor H, also injected with antigen 8 weeks before transfer to each of 20 unilaterally adrenalectomized mice, 10 of the Rockefeller Institute strain and 10 C mice. All were challenged with the same antiserum used in the preceding tests, half under anesthesia and half without it. Twelve of the 20 mice that received the donor's liver showed either weak positive EVR or positive objective signs of Phase I of reversed passive anaphylaxis (plus signs in columns 10 and 12, respectively, in Table 1). Although these positive reactions were definitely weak, 2 of the mice—one of them a mouse of the Rockefeller Institute strain, the other a C mouse—when challenged without anesthesia showed signs of Phase II of gross anaphylaxis and also swelling of the lips, tongue, eyes, and forepaws,—the urticaria-like reaction already described (asterisk in column 12).

Eight more similarly treated recipients, when given the same liver tissue and challenged—half under anesthesia and half without it—with antigen, for evidences of transferred antibody, yielded negative findings (minus signs in columns 10 and 12).

Clearly, the second series of tests corroborated the findings of the first and indicated the detection of antigen persisting in the transferred donor's liver for 8 weeks. The positive reactions were definitely weak. Consequently it seemed likely that materials taken from donor rabbits after intervals longer than 8 weeks would yield, at best, only dubiously positive or negative results. Such findings would be of little value since they could point only to an unverifiable conclusion that faint traces of antigen too small to be detected might continue to persist in the liver for further indeterminate periods of time. Accordingly attempts were abandoned to search the livers of donor rabbits for antigen persisting longer than 8 weeks.

The Persistence of the Antigen in Rabbits Compared with That Occurring in Mice.—In the previous paper (1) bovine γ-globulin, injected once into mice, was found by mouse transfer tests persisting in the blood for 8 weeks and in the liver for 14 weeks. In the present work this antigen persisted in the blood of rabbits for 4 weeks in easily detectable amounts, and it was demonstrated, in traces, in the blood of one rabbit after 6 weeks. In the liver the antigen was still present after 8 weeks, but by the present means it seemed unlikely that further persistence could be demonstrated. From this it seems fair to conclude that, in the rabbit, which forms antibody well, the persistence was not as long as in the mouse, which forms antibody poorly. Nevertheless, as already stressed, the antigen, injected once only, persisted in the liver after 8 weeks when no circulating antibody could be detected.

DISCUSSION

As already indicated in this and in a previous paper (1), the EVR test suffers from an undesirable feature. The observer, looking through the microscope at the blood flow and the minute vessels of the ears of the recipient mice during
challenge, must distinguish the specific anaphylactic responses from non-specific "injection reactions" or other non-specific physiological responses that may appear from time to time. The occurrence of these non-specific reactions detracts from the general usefulness of the procedure, since it can hardly be expected to serve workers who have not become thoroughly familiar with the variety of vascular changes that can take place in the ears of intravenously injected mice. During the present work, whenever vascular reactions appeared that could not be clearly distinguished either as specific anaphylactic responses or typical "injection reactions," it became necessary to discard all detection tests with the material at hand and to repeat the experiment from the beginning. Since the "injection reactions" could not be entirely avoided they were reduced to a minimum by exercising the precautions already described. It was noted that the tests for transferred antibody, carried out by giving challenging injections of solutions of bovine $\gamma$-globulin instead of serum, were singularly free from "injection reactions." So too in other work, injections of various protein solutions, instead of whole serum have not been accompanied by "injection reactions." Experiments now being elaborated are showing that much difficulty in carrying out EVR tests can be avoided by challenging the test animals with solutions of the globulin precipitate obtained from immune serum, instead of using whole serum. In a number of experiments, made up to this time, to test the sensitivity of the reactions carried out in this way there have been no "injection reactions."

The new procedure reported above—the observance by several workers, of the gross behavior of mice challenged without anesthesia—produced a welcome technique for running parallel tests to supplement the findings obtained by EVR tests.

Urticarial swelling of the face and edema of the paws and lower legs of recipient mice offered another welcome objective sign, especially when it appeared in anesthetized animals which could not have brought it about by scratching. Observations of the circulation of blood in the ears of the recipient mice that showed the sign when under anesthesia, indicated that it appeared when the slowing of peripheral flow—one of the positive signs of EVR (1)—was moderate and prolonged, so that on recovery, about 12 to 20 minutes after challenge, there was a marked congestive, reactive hyperemia favorable for the formation of the local edema. When shock was severe enough to produce circulatory stasis, the phenomenon was not seen. It failed to appear, too, if the reaction was too mild to be followed by hyperemia (1).

The finding that the loss of one or both adrenals enhances the sensitivity of mice to reversed passive anaphylactic shock, stems from the work of others (25–27), who showed that the procedures increase the sensitivity to ordinary active or passive anaphylaxis. In 1947 Murphy and Sturm (52) found rabbits deprived of both adrenals capable of forming more antibody than intact
animals. Others have shown that excess adrenocortical stimulation by injections of cortisone (53-58) decreases antibody formation. However, it is difficult to see how differences in antibody formation could have enhanced the anaphylactic sensitivity of those recipient mice that were given antigen, or antigen-containing materials, by intraperitoneal injection and challenged by subsequent injections of pre-formed antibody only 48 hours later. Work is now in progress to determine whether or not certain physiological effects may throw some light on the question. Discussion of the matter can best be postponed until the completion of that work.

So far the findings obtained by the mouse transfer tests point to conclusions that are surprising to many workers, and quite unacceptable to some. Why do the mouse transfer tests apparently detect antigen in liver tissue, when, as is well known from the work of many others, methods of extraction fail to detect any? Does the recipient mouse absorb antigen better than man can extract it? This may readily be the case since antigen, within or adsorbed upon cells, can be carried down with the tissue debris when tissue extracts are centrifuged to yield clear supernatant fluids for precipitin tests.

On the other hand, can it be that antigen, or antigen-like material, persisting in the blood and tissues, is capable only of rendering the test animals anaphylactically sensitive and not able to take part in antibody formation? Work showing that this is not the case will be reported fully in a subsequent paper. To anticipate briefly; in that work repeated transfers of liver tissue with certain adjuvants, from donor mice injected several weeks before with bovine \( \gamma \)-globulin, to recipients has rendered the latter sensitive to active anaphylactic shock when reinjected with the same antigen 3 weeks after the last transfer. Under these circumstances the transferred liver tissue, presumably containing antigen, had obviously harbored it in such a form that the recipients formed antibodies which reacted 3 weeks later when challenged with the original antigen.

The matter is of importance because the present findings have indicated also that antigen persisted in the liver all through the period in which antibody circulated in the blood, since antigen could still be found in that organ at the 8th week after circulating antibody was no longer demonstrable. This state of affairs is not incredible since a similar situation has been found by Olitsky and his coworkers with Western equine encephalitis virus (59). This virus can persist in mice for weeks, even when there is much circulating antibody. Later, after the disappearance of circulating antibody, it may initiate the disease. In the experiments reported here, why was there no demonstrable antibody in the blood if antigen was still present in the body? If the antigen was held and destroyed by the cells which captured it, one would not expect to find further formation of circulating antibody. If, on the other hand, antigen, persisting in the liver, was released, after antibody was no longer detectable,
one would have expected to find further antibody formation; unless, which might well be, the amounts of antigen liberated were too small to elicit the formation of antibody in sufficient quantity either to appear in the circulation or to be detected.

However this may be, a simpler solution to the problem presents itself. Is it not possible that an injected, protein antigen, taken up into reticulo-endothelial cells such as Kupffer cells, persists not as a simple antigen but as an antigen-antibody complex, which, if extracted from the tissue, fails to give a precipitin reaction? When injected into mice the complex may be dissociated, and the antigen liberated to sensitize the injected mouse. That something of the sort can occur is shown by the findings, here presented, from one of the experiments with serum from donor C, taken 4 weeks after injecting the antigen into the rabbit. Precipitin tests showed (Table I, column 3) the presence of antibody only, whereas the mouse transfer tests were positive for both antigen and antibody (Table I, columns 5 and 7). Work of other authors (60-62) indicates that complexes of the sort, failing to give precipitin reactions, may be present in immune sera or in tissues. Work is now going forward upon the question.

SUMMARY

A sensitive biological test has been used to detect the persistence of minute traces of a foreign protein, bovine \( \gamma \)-globulin, in the blood and livers of rabbits intravenously injected with it, as an antigen. At various intervals after injecting these rabbits (donors) serum or liver tissue was transferred from them to the peritoneal cavities of normal or unilaterally adrenalectomized mice (recipients) with the aim of rendering the latter hypersensitive to the antigen that might be persisting in the transferred materials; a state of affairs detectable, 2 days later, by the appearance of signs of reversed passive anaphylaxis when the recipient mice were intravenously challenged with a strong antibovine \( \gamma \)-globulin rabbit serum.

The protein persisted in the blood of the donor rabbits, in readily demonstrable amounts for 1 month, and in the blood of one animal, in minute traces, or as long as 6 weeks. It was detectable in the livers for 8 weeks.

The persistence of bovine \( \gamma \)-globulin in rabbits, which form circulating antibodies to it well, is not as long as that in mice, which form antibodies to it poorly, since in previous work with the mouse the antigen was found (1) in the blood after 8 weeks and in the liver for 14 weeks. Nevertheless the antigen persists in the rabbit much longer than is generally supposed. Indeed it can be found in the liver all through the period in which circulating antibody is demonstrable in the blood. Explanations for the phenomenon have been suggested. Its significance in relation to the mechanisms of antibody formation is obvious.
BOVINE \(\gamma\)-GLOBULIN INJECTED AS ANTIGEN

BIBLIOGRAPHY

1. McMaster, P. D., and Kruse, H., J. Exp. Med., 1951, 94, 323.
2. Kruse, H., and McMaster, P. D., J. Exp. Med., 1949, 90, 425.
3. Well, R., J. Med. Research, 1912-13, 27, 497.
4. Well, R., J. Med. Research, 1914, 30, 350.
5. Well, R., J. Med. Research, 1914, 30, 357.
6. Longcope, W. T., and Rackemann, F. M., J. Exp. Med., 1918, 27, 341.
7. Longcope, W. T., and Mackenzie, G. M., Proc. Soc. Exp. Biol. and Med., 1920, 17, 133.
8. Mackenzie, G. M., and Leake, W. H., J. Exp. Med., 1921, 33, 601.
9. Pressman, D., and Keighley, G., J. Immunol., 1948, 69, 141.
10. Pressman, D., Hill, R. F., and Foote, F. W., Science, 1949, 109, 65.
11. Pressman, D., J. Immunol., 1949, 68, 375.
12. Pressman, D., and Eisen, H. N., J. Immunol., 1950, 64, 273.
13. Eisen, H. N., and Pressman, D., J. Immunol., 1950, 64, 487.
14. Pressman, D., Eisen, H. N., Siegel, M., Fitzgerald, P. J., Sherman, B., and Silverstein, A., J. Immunol., 1950, 65, 559.
15. Pressman, D., Cancer, 1949, 2, 297.
16. Crampton, C. F., and Haurowitz, F., Science, 1950, 112, 300.
17. Haurowitz, F., Crampton, C. F., and Sowinski, R., Fed. Proc., 1951, 10, 560.
18. Crampton, C. F., Reller, H. H., and Haurowitz, F., Proc. Soc. Exp. Biol. and Med., 1952, 80, 448.
19. Haurowitz, F., and Crampton, C. F., J. Immunol., 1952, 68, 73.
20. Crampton, C. F., and Haurowitz, F., J. Immunol., 1952, 69, 457.
21. Crampton, C. F., Reller, H. H., and Haurowitz, F., J. Immunol., 1953, 71, 319.
22. Ingraham, J. S., J. Infect. Dis., 1951, 89, 109.
23. Stevens, K. M., J. Exp. Med., 1953, 97, 247.
24. Schiller, A. A., Schayer, R. W., J. Gen. Physiol., 1953, 36, 489.
25. Weiser, R. S., Golub, O. J., and Hamre, D. M., J. Infect. Dis., 1941, 68, 97.
26. Halpern, B. N., and Wood, D., Compt. rend. Acad. sc., 1950, 230, 138.
27. Dougherty, T. F., Pituitary Adrenal Function, (R. C. Christman, editor), Washington, D. C., American Association for the Advancement of Science, 1950, 79.
28. McMaster, P. D., J. Exp. Med., 1941, 74, 29.
29. Braun, H., Münch. med. Woch., 1909, 56, 1880.
30. Braun, H., Z. Immunitttsforsch., Orig., 1909, 4, 590.
31. Schütz, W. H., and Jordan, H. E., J. Pharmacol. and Exp. Therap., 1910-11, 2, 375.
32. Ritz, H., Z. Immunitttsforsch., Orig., 1911, 9, 321.
33. von Sarowskii, Z. Immunitttsforsch., Orig., 1913, 17, 577.
34. Schiemann, O., and Meyer, H., Infektionskrankh., 1926, 306, 607.
35. Bowdon, K. L., Proc. Soc. Exp. Biol. and Med., 1937, 36, 340.
36. Mayer, R. L., and Brousseau, D., Proc. Soc. Exp. Biol. and Med., 1946, 63, 187.
37. Nelson, C. T., Fox, C. L., Jr., and Freeman, E. B., Proc. Soc. Exp. Biol. and Med., 1950, 75, 181.
38. Wheeler, A. H., Brandon, E. M., and Petrenco, H., J. Immunol., 1950, 65, 687.
39. Einbinder, J. M., Nelson, C. T., and Fox, C. L., Jr., *Fed. Proc.*, 1954, **13**, 490.
40. Spoerlein, M. T., and Margolin, S., *Fed. Proc.*, 1954, **13**, 408.
41. Olitsky, P. K., and Lee, J. M., *Proc. Soc. Exp. Biol. and Med.*, 1954, in press.
42. McMaster, P. D., and Kruse, H., *J. Exp. Med.*, 1949, **89**, 583.
43. Parfentijev, I. A., *Yale J. Biol. and Med.*, 1950, **23**, 28.
44. Solotorovsky, M., Porter, C. C., and Silber, R. H., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74**, 313.
45. Solotorovsky, M., and Winsten, S., *J. Immunol.*, 1953, **71**, 296.
46. Landsteiner, K., and van der Sheer, J., *J. Exp. Med.*, 1932, **56**, 399.
47. Anderson, H. C., and McCarty, M., *Am. J. Med.*, 1930, **8**, 445.
48. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1932, **56**, 555.
49. McMaster, P. D., and Hudack, S. S., *J. Exp. Med.*, 1932, **56**, 417.
50. McMaster, P. D., unpublished data.
51. Sternberger, L. A., Maltaner, F., and De Weerolt, S., *J. Exp. Med.*, 1953, **98**, 460.
52. Murphy, J. B., and Sturm, E., *J. Exp. Med.*, 1947, **86**, 303.
53. Fischel, E. E., Le May, M., and Kabat, E. A., *J. Immunol.*, 1949, **61**, 89.
54. Fischel, E. E., *Bull. New York Acad. Med.*, 1950, **26**, 255.
55. Björneboe, M., Fischel, E. E., and Stoerk, H. C., *J. Exp. Med.*, 1951, **93**, 37.
56. Stoerk, H. C., and Solotorovsky, M., *Am. J. Path.*, 1950, **26**, 708.
57. Germuth, F. G., Jr., and Ottinger, B., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74**, 815.
58. Robinson, H. J., and Smith, A. L., *Ann. New York Acad. Sc.*, 1953, **56**, 757.
59. Olitsky, P. K., Schlesinger, R. W., and Morgan, T. M., *J. Exp. Med.*, 1943, **77**, 359.
60. Garvey, J. S., and Campbell, D. H., *J. Immunol.*, 1954, **72**, 131.
61. Garvey, J. S., and Campbell, D. H., *Fed. Proc.*, 1954, **13**, 493.
62. Sternberger, L. A., *Fed. Proc.*, 1954, **13**, 513.
EXPLANATION OF PLATE 45

Figs. 1 to 3. An objective phenomenon of reversed passive anaphylaxis. Three views of a pair of unilaterally adrenalectomized mice both of which received, 48 hours previously, 0.5 ml. of serum from a rabbit 4 weeks after it had been injected with bovine $\gamma$-globulin. The “test” mouse—on the right in Figs. 1 and 3 and above its “control” in Fig. 2—challenged with anti-bovine $\gamma$-globulin rabbit serum, shows swelling of the lips, face and paws, absent in the control mouse, which was challenged with normal rabbit serum. Since both animals were challenged under anesthesia the swelling was not brought about by scratching. For further details see the text. Figs. 1 and 2, $\frac{3}{4}$ natural size. Fig. 3, $1\frac{1}{2}$ natural size.
(McMaster et al.: Bovine γ-globulin injected as antigen)