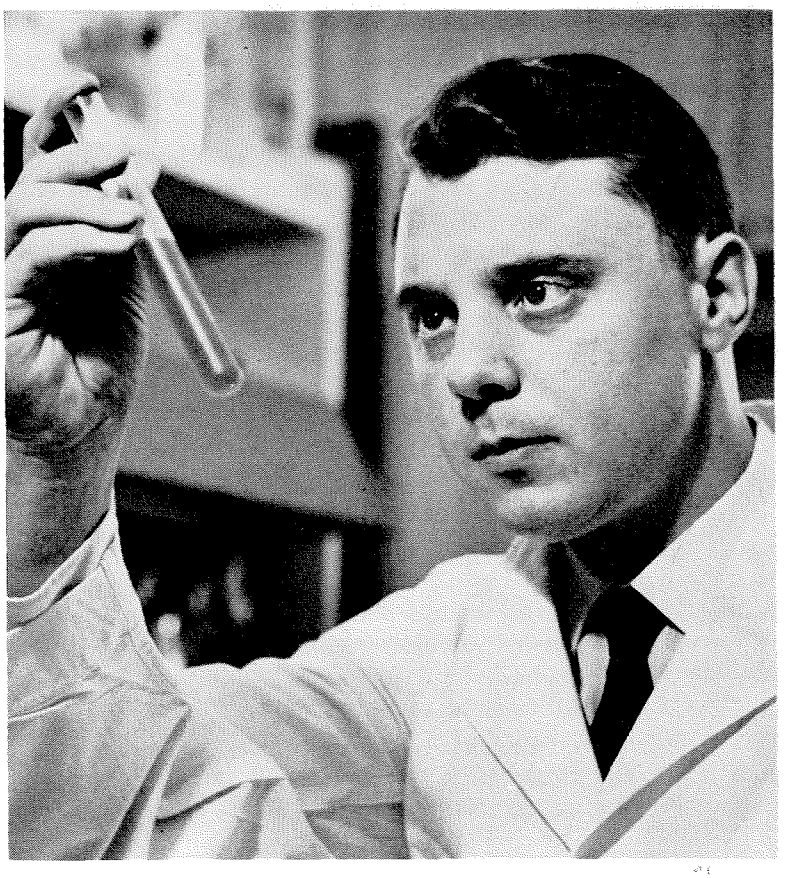


by Dieter H. Sussdorf

The Appendix — New Facts About a Lowly Organ

Most people consider the appendix an obsolete, utterly useless organ. It can, however, be an interesting research subject for the immunologist. And — in certain animals, at least — it may even serve some physiological purpose.

Our research on the appendix did not begin with an experiment solely devoted to this organ. It began with an evaluation of the importance of various organs in antibody formation — a process which insures the defense of the body against the attacks of many microorganisms. Antibodies are proteins which are made by certain cells in certain organs in response to the introduction of foreign substances called antigens into the body. Antibodies have the capacity to combine with the antigens which stimulated their manufacture. Since invading microorganisms are made up of, and produce, substances foreign to the animal they attack, the antigen-antibody combination may result in the destruction of the bacterial cell, and in rendering harmless such bacterial products as toxins.

Where in the body are antibodies made? It has been known for many years that organs involved in this process are rich in lymphatic tissue — a tissue capable of producing a species of white blood cells, the lymphocytes. Organs of major interest in this group have been the lymph nodes and the spleen. Another organ that has been implicated is the liver. Although it contains only a relatively small amount of lymphatic tissue, it is very active in the synthesis of certain blood proteins.

One way to study the importance of these organs in antibody production is to remove them surgically from an experimental animal, either singly or in various combinations. After surgery, the animals are injected with a foreign protein. The amount of antibody formed in response to this injection is followed over a period of time. If a surgically removed organ is significantly involved in the manufacture of antibodies, the antibody response should be depressed.

A second method of study is based on the use of X rays. Because lymphatic tissue is more susceptible to the destructive effects of this radiation than are most other tissues, an animal's antibody response can

Obsolete? Useless? Not to the immunologist, who has found the appendix to be a particularly interesting research subject.

be greatly delayed and depressed by exposing its body to X rays. If, however, an organ involved in antibody formation is protected with a lead shield during irradiation, antibody levels should be higher than in unprotected animals.

Experiment I

The first of this series of experiments was begun by the author and Dr. Laurence R. Draper at the University of Chicago, under the sponsorship of the U.S. Atomic Energy Commission. The work was then continued at the Argonne National Laboratory and completed at Caltech in the laboratory of Dr. Dan Campbell, professor of immunochemistry.

Our work on the appendix began when we became interested in organs supposedly involved in antibody synthesis. However, an additional interest existed. Since the end of World War II there has been an intense research effort concerned with the effects of atomic radiations on the mammalian body, and with the protection of radiation-exposed animals against these effects. The immunologist's interest in radiation research derives from the fact that one cause of radiation death is the breakdown of the body's defense system against infection, and the resulting invasion of the body by bacteria – especially those residing in the intestinal tract.

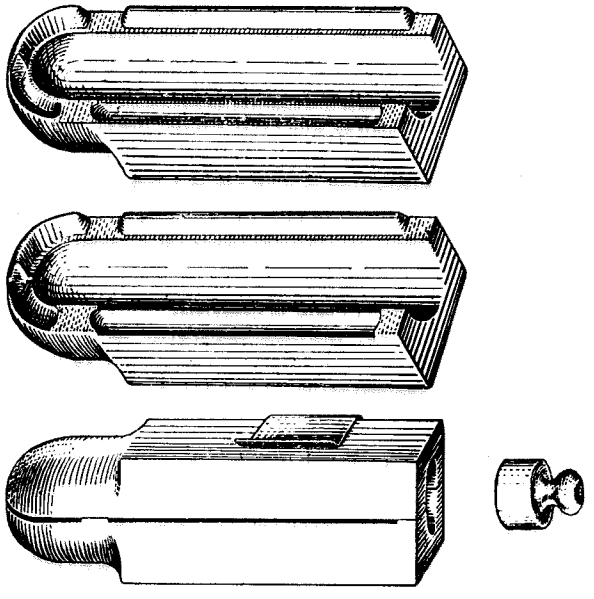
By designing an experiment which involved the shielding of antibody-forming organs of an experimental animal during x irradiation, we hoped to obtain information on both problems: that of the importance of the organ studied as an antibody former, and that of the protection of the immune system against radiation effects.

For our study, we decided on the rabbit as the experimental animal, and we selected the spleen, the appendix, and the liver as the organs to be investigated.

In the rabbit, the spleen is a dark red organ, about 1½ inches long and ¼ inch thick, located near the stomach. It consists of two major tissue components which are present in about equal amounts: the "white pulp" which is strictly lymphatic tissue, and the blood-storing "red pulp." The appendix (more accurately, the *vermiform* appendix) is the end portion of a blind intestinal pouch located at the juncture of the small and large intestines. The rabbit appendix is about 3 inches long and 1/3 inch thick, and its wall is very rich in lymphatic tissue similar to that found in the tonsils. The rabbit appendix is approximately the same size as the human appendix – which means that the appendix in an adult rabbit is relatively about 30 times larger than in an adult man. This enormous difference in relative size does not justify a direct application of our experimental findings to man.

In order to protect the organs of interest to us during irradiation, special lead shields had to be designed and manufactured. They had to meet three major requirements; (1) they should cover the organ only and no other part of the body, (2) they should provide an opening for the blood vessels attached to the organ, and (3) they should be free of any X ray leaks. The lead shield used to protect the spleen or appendix is shown below. The shield consisted of two halves which, when joined, were held together by a steel clamp. The spleen or appendix rested in the hollow center of the shield, while the blood vessels passed freely through a baffle on its side. The open end of the shield, which allowed for the passage of the intestine in the case of appendix shielding, was closed with a lead plug when the spleen was protected.

Because of the anatomy of the liver and the shield requirements we had set forth, we were unable to



The lead shield used to protect the spleen or appendix of the rabbit during x-irradiation.

design a shield which could accommodate the whole liver. The final version of this shield was, in principle, similar to that for the spleen and appendix, except that it was much larger and contained only about ¾ of the liver.

The rabbits were divided into five series. Each one of three series was x irradiated with one of the three organs shielded. The fourth series was totally irradiated (no shield was applied during irradiation) and the fifth series remained unirradiated. The shielding of an organ involved giving the animal an anesthetic, making an incision, applying the shield to the organ, and placing the anesthetized animal under the X ray beam. After irradiation, the shield was removed and the incision closed. The X ray dose was 500 roentgens, a dose which causes a marked delay and depression of antibody formation in the unprotected rabbit. If the animal survives, the antibody-producing mechanism requires about four weeks to recover.

On the day following irradiation, our rabbits were injected intravenously with an antigen, red blood cells of the sheep. The antibodies formed against these cells have the capacity to lyse them, i.e., to cause the release of hemoglobin from the cell interior. Therefore, in order to measure the concentration of antibody in a sample of blood taken from one of the injected rabbits, we determine how much serum is required to lyse a known number of sheep red cells. The smaller the amount of serum required, the greater is the antibody content of the blood sample.

A surprising observation

We made a surprising observation upon examination of our experimental data. Shielding the spleen or liver during irradiation resulted in an antibody response about halfway between that of the totally irradiated and the unirradiated rabbits (indicating about 50 percent protection). However, the response was practically normal in the appendix-shielded animals (indicating almost complete protection).

This finding was unexpected because the spleen has always been considered a major site of antibody production when the antigen is introduced via the intravenous route, while no contribution of the appendix could be demonstrated. These conclusions are based on experiments in which the spleen or appendix was removed surgically before the injection of antigen. If the spleen is absent, antibody production is greatly delayed and depressed. If the appendix is removed, antibody formation remains unaffected. Thus, an apparent paradox had to be resolved: although the appendix does not seem to participate in the antibody response, shielding the organ during irradiation results in almost complete protection of the response. If we assume that the spleen is the most important antibody contributor and that the recovery of the antibody-producing mechanism in an x irradiated rabbit is related to the recovery of this organ, the following question arises: Could, in some way, the shielded appendix accelerate the recovery of the spleen from radiation damage?

Experiment II

The question of the effect of the appendix on the recovery of the spleen immediately suggested a second experiment. We would have to study the changes in that tissue component of the spleen which is most likely associated with antibody production. We learned earlier that this component is the white pulp. If we assume that, within certain limits, the amount of white pulp in the spleen is related to the organ's antibody-producing capacity, we could follow the changes in this capacity by measuring the amount of white pulp at intervals after irradiation.

Experimentally, this approach required first the preparation of an appendix-shielded and a totally irradiated series of rabbits as outlined in Experiment I. Then, at intervals after irradiation covering a period of up to 67 days, the spleens were removed from groups of 3 to 4 animals in each series. The organs were cut into sections, mounted on glass slides and stained. With the aid of a projector, an enlarged image of the spleen was projected on paper, on which the outline of the spleen and of the areas representing the white pulp were traced. From the proportional sizes of these areas, the amount of white pulp could then be calculated.

The recovery curves we obtained for the splenic white pulp confirmed our earlier supposition: the antibody-producing tissue in the spleen regenerated much faster in the appendix-shielded than in the unprotected animal. Until about the fourth day after irradiation, the white pulp virtually disappeared in both series of rabbits. From then on, differences between the recovery rates became apparent. While we did not find normal amounts of white pulp in the totally irradiated animals until 23 days after irradiation, normal amounts were already present on the 8th day in the appendix-protected rabbits. Apparently, the shielded appendix caused the spleen to develop, in very short time, enough antibody-forming tissue to insure a virtually normal antibody response.

What was the nature of the effect of the appendix on the spleen? After having examined the spleens of the appendix-shielded rabbits microscopically, we began to believe that the lymphoid cells appearing in the spleen after the 4th day could not all have arisen locally, since very little cell division occurred in the organ at that time. The cells apparently came from somewhere else — perhaps from the appendix.

Experiment III

With our third experiment, we intended to examine the possibility that lymphoid cells from the shielded appendix repopulated the spleen, causing its accelerated recovery. To demonstrate this cell migration,

it seemed essential to mark the cells of the appendix in some manner and to look for these cells in the spleen. Fortunately, a recent development in radiobiology provided the tools for this experiment.

By attaching a radioactive atom (tritium) to a substance (thymidine) which is used by the cell nucleus as a building block, a radioactive cell label can be made. Labeling of cells occurs within a few hours after the injection of the tritium-labeled thymidine, when all the cells engaged in the synthesis of nuclear material pick up the labeled compound and deposit it in the nucleus.

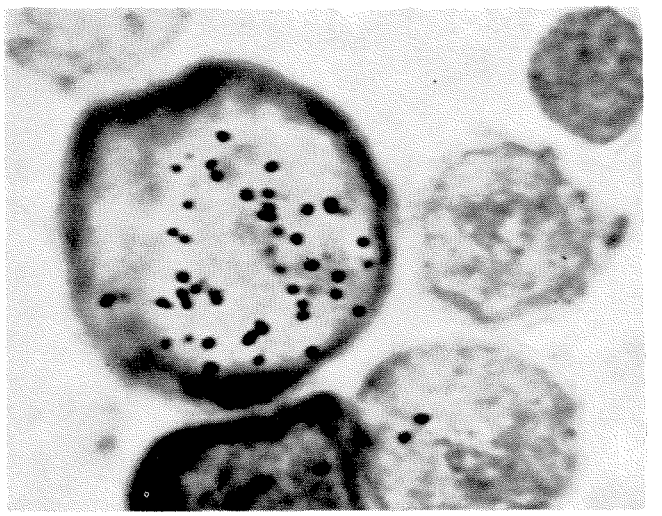
Detecting radioactive cells

We can detect these radioactive cells by preparing stained slides as we did for the spleen and covering the slides with photographic film. After about 6 weeks, the emulsion immediately above each labeled nucleus will have been exposed by the radioactive emission from the tritium. If the film is then developed (actually both the slide and the attached film are processed), and the slide examined under the microscope, tiny black dots can be seen overlying the nucleus of a labeled cell (right). This radioactive label represents a perfect marker since it remains with the cell until it dies. If the cell divides, each of its daughter cells receives ½ of the label. In this way, the label is diluted progressively until it becomes undetectable.

One major feature made this technique especially useful to us: the tritium-labeled thymidine is incorporated only by cells preparing for cell division. Since X rays inhibit the division of surviving cells, and since only the shielded appendix should contain an appreciable number of intact cells, most of the label, we hoped, would be taken up by the appendix.

This hope was borne out by our third experiment. Within a few hours after the injection of the labeled compound into a group of x irradiated, appendix-shielded rabbits, over 20 percent of the lymphoid cells of the appendix were labeled, while practically no such cells were found in the spleen. To make sure that among the lymphoid organs only the appendix contained a significant number of tagged cells, we also examined a lymph node. But here, too, no labeled cells were present.

Then our main expectation was realized. On the day following the first examination, labeled cells appeared in the spleen. A temporary decrease of the percentage of these cells occurred in the appendix at the same time. To us, these observations furnished a very good support for the repopulation hypothesis. The intact lymphoid cells in the appendix apparently left this organ shortly after irradiation, and implanted in those organs where lymphatic tissue was destroyed by the X rays. Once implanted, the cells began to proliferate, bridging over the time gap between X ray exposure and the return of the exposed but surviving cells to normal.



A lymphoid cell of the appendix carrying a radioactive marker (represented by the black dots).

The observation that shielding the spleen gave only 50 percent protection still had to be explained. There is some evidence that lymphocytes proliferate better in an environment rich in cellular breakdown products. This type of environment existed in the spleen exposed to x irradiation and was encountered there by the large number of cells coming from the shielded appendix. Some cell destruction occurs even in a shielded spleen, but the ratio between the amount of breakdown products and the number of surviving cells may not be as favorable.

Summing up

The appendix in man is, relative to body size, about 30 times smaller than in the rabbit. Consequently, this organ is probably immunologically insignificant in the human, but may fulfill a defense purpose in the rabbit. We failed to demonstrate any contribution of the appendix in the unirradiated rabbit to the antibody response – probably because the organ did not receive enough of the intravenously injected antigen. Nevertheless, as we have seen, the lymphoid cells of this organ are capable of transforming into antibody-producing cells if they meet the proper conditions. We may generalize that, irrespective of their place of origin, lymphocytes can migrate to and implant in any part of the body, and develop there into antibody-forming cells if they encounter antigenic substances.

The shielding experiments permit a speculation on a quite different subject – that of cancer therapy. Certain forms of cancer call for therapy involving high doses of radiation administered to the whole body. One of the problems associated with this treatment is the subsequent breakdown of the body's defense system against infection. According to our experimental results, it might be possible to minimize this complication by shielding small areas rich in lymphatic tissue during the radiation treatment.