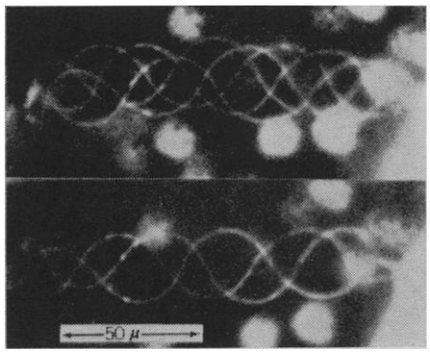

Fig. 1. Multiple-flash photomicrographs of the posterior flagellum of *Ceratium*. The posterior end of the cell body is visible at the right edge of the photographs. Flash rate, 25 per second.

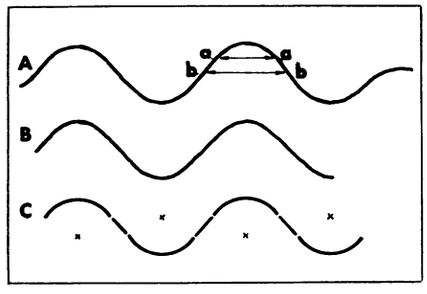

Fig. 2. *A*, Curves traced from a photograph of a flagellar waveform; *B*, curve constructed from the function $y = \sin x$, with wavelength and amplitude matched to the waveform in *A*; *C*, curve constructed of circular arcs and straight lines to match the waveform in *A* as closely as possible.

ments was 0.025; the difference is significant well beyond the 0.001 level of probability. The deviations from a sine wave are not therefore simply random irregularities, but indicate that the regular form of the wave is not sinusoidal.

The photographs also reveal that the bent regions of a flagellum are usually circular arcs in which the curvature is constant throughout the bend. Wave patterns which fit the flagellar waveforms almost exactly can be constructed from circular arcs and straight lines as shown in Fig. 2*C*. In this example, all the arcs were drawn with the same radius, indicating that the amount of bending in the bent regions was constant over most of the length of the flagellum. Periodic active bending must occur all along the flagellum to maintain the constant amplitude of the bending waves (*1*, *4*).

The difference between the sine wave and the wave constructed with circular arcs and straight lines is small and does not seriously affect the results of integration obtained for estimating the propulsive effect and energy expenditure, so that previous conclusions based on sine wave calculations (*5*) are not invalidated. The significance of the difference lies in its implications about the mechanism of wave generation.

If active bending is generated by short, mechanically independent elements distributed along the flagellum (*1*, *4*), the shape of the waveform is determined by, and therefore provides information about, the control mechanism which coordinates the activity of the bending elements. If the control mechanism involves transmission of information along the flagellum to trigger active bending, the wave pattern composed of circular arcs and straight lines could be generated by a simple on-or-off control of bending. This would appear to require much less information than would have to be transmitted to generate a sine wave by controlling the amount of bending or the bending moment.

Machin has proposed (see *4*) that active bending is triggered by bending induced by the activity of other regions of the flagellum. This proposal was originally formulated in terms of a linear system generating sinusoidal waves. In a recent paper (*6*), Machin has demonstrated that this model requires non-linear behavior of the active bending elements in order to generate the propagated, unidirectional waves which appear on flagella. The on-or-off character of the waveform composed of circular arcs and straight lines clearly implies non-linear activity of the bending elements, and increases the plausibility of Machin's model (*7*).

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4 October 1963

Contamination of Commercial Rabbit Albumin Preparations by Bovine Albumin

Abstract. *Seven commercial rabbit serum albumin preparations obtained from two different sources were found to contain bovine serum albumin. The extent of contamination in three representative samples varied from 0.35 to 0.54 percent.*

During preliminary studies in which rabbit serum albumin (RSA) was employed as a hapten carrier for rabbit immunizations, certain findings suggested that the commercial preparations in use contained bovine serum albumin (BSA). This prompted the detailed examination of one such preparation, and a survey of other lots for similar contamination. In all, seven commercial RSA samples prepared by Cohn fractionation, and one made in this laboratory (RSA La Jolla) were the method of Schwert (*1*), were tested. All except the one prepared in this laboratory (RSA La Jolla) were contaminated, as determined by quantitative precipitation, agar-gel diffusion, and inhibition of the reaction between I^{131}-labeled BSA and rabbit antiserum to BSA.

One-half milliliter of a pooled hyperimmune rabbit antiserum to BSA was absorbed with 234 mg of RSA No. 51

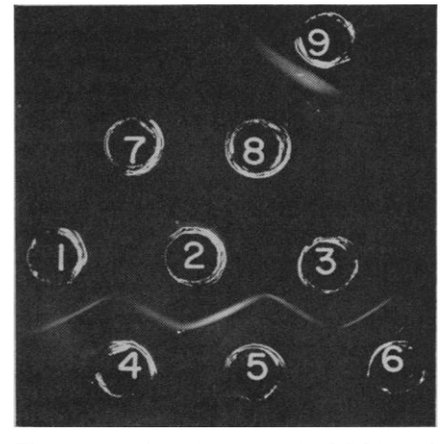

Fig. 1. Reactions in agar gel of BSA and commercial RSA No. R51 with antiserums to bovine serum albumin and commercial rabbit serum albumin. The continuity of the wavy line between wells 1 and 4, 2 and 5, and so forth, denotes a reaction of identity. Well 1 and 3: rabbit "anti-commercial RSA"; well 2: hyperimmune rabbit anti-BSA; well 4 and 6: commercial RSA No. R51 (160 μg N); well 5: BSA (2 μg N); well 7: RSA (La Jolla) (160 μg N); well 8: RSA (La Jolla) (10 μg N); well 9: bovine anti-RSA.

for 1 hour at 37°C and 48 hours in the cold. A heavy precipitate formed which was removed by centrifugation. The supernatant fluid was tested by the quantitative precipitin method with increasing amounts of BSA, along with a control of the original unabsorbed antiserum. The control serum was found by these procedures to contain 1275 μg of antibody (nitrogen) to BSA per milliliter, while with the RSA-absorbed sample, no precipitate was formed with any of the amounts of BSA which were added (2.4 to 30.5 μg of BSA nitrogen).

Rabbits were immunized with two injections of incomplete Freund's adjuvant containing 150 mg of commercial RSA preparation administered 2 weeks apart. These animals produced antibody to BSA, as determined by ammonium sulfate precipitation of combined I^{131}-BSA and antibody to BSA, but made no detectable antibody to RSA. One such serum contained 324 μg of antibody (nitrogen) to BSA, as determined by quantitative precipitin tests.

Figure 1 shows reactions of identity in agar gel, between BSA and a commercial RSA preparation, when tested against rabbit antiserums to BSA and to commercial RSA. Uncontaminated RSA (RSA La Jolla) failed to precipitate with either antiserum, but did react with bovine antiserum to RSA. Similar studies with rabbit antiserum to BSA showed strong reactions of identity between BSA and all seven commercial RSA preparations, with no evidence of a cross reaction.

The inhibition studies were based on techniques described by Farr (*2*). Serial dilutions of a rabbit antiserum to BSA were incubated with I^{131}-labeled BSA (0.05 μg N) in the presence of a large excess of unlabeled commercial RSA preparation. After an overnight incubation at 25°C, the antigen bound to antibody globulin was precipitated by addition of an equal volume of saturated ammonium sulfate, washed with 50 percent saturated ammonium sulfate, and detected by counting in a scintillation (NaI crystal) counter. As a result of competition between the I^{131}-labeled BSA and the unlabeled BSA in the RSA samples, it took more antiserum to bind a given amount of labeled BSA in the presence of contaminated RSA than in controls lacking RSA. The dilution of antiserum necessary to bind 40 percent of the 0.05 μg of nitrogen in the labeled BSA, in the presence of commercial RSA (75 μg N), was determined for each sample, and compared with the dilution of antiserum required to bind 40 percent of the labeled BSA alone, as shown in Table 1. From 52 to 89 percent inhibition of the reaction between I^{131}-BSA and antibody to BSA was brought about by commercial RSA (75 μg N) at 1500 times the concentration of labeled BSA present. For comparison, there was no significant inhibition by uncontaminated RSA (750 μg N) prepared here and designated RSA La Jolla; there was 99.8 percent inhibition by BSA (50 μg N).

In an attempt to determine the amount of BSA in representative commercial preparations of RSA, the following procedure was used. From preliminary testing, a dilution of antiserum to BSA was found which was

Table 1. Blocking of reaction between I^{131}-BSA and antiserum to BSA by commercial RSA preparations.

Unlabeled blocking antigen		Blocking antigen concentration*	Blocking activity† (%)
RSA (7501)‡	fraction V	1500	52
RSA (7503)‡	fraction V	1500	68
RSA (3517)§	fraction V	1500	83
RSA (R51)‡	fraction V	1500	84
RSA (3470)§	recrystallized	1500	72
RSA (5)‡	recrystallized	1500	77
RSA (4)‡	recrystallized	1500	89
RSA (La Jolla)		1500	3.4
RSA (La Jolla)		15,000	1.1
BSA (Armour recrystallized)		1000	99.8

* Ratio of concentration of blocking antigen to that of I^{131}-BSA. † [(Titer without blocking antigen — titer with blocking antigen)/titer without blocking antigen × 100]. ‡ Purchased from company A. § Purchased from company B. The source can be obtained from the author.

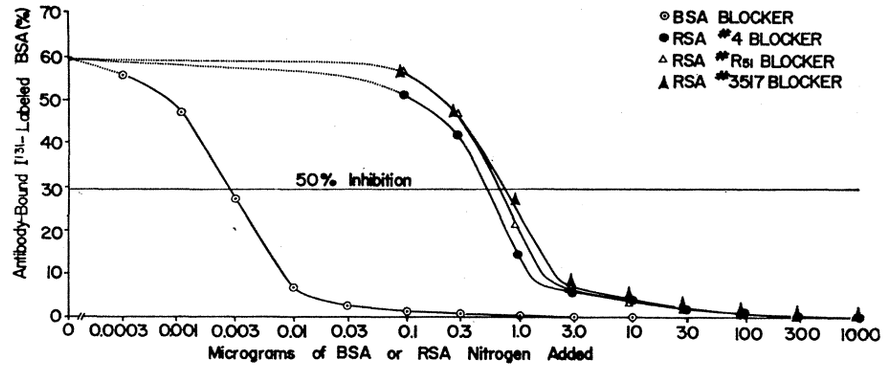

Fig. 2. Inhibition of reaction between I^{131}-BSA and antibody to BSA, by commercial RSA preparations and by BSA. Ordinate: percent of I^{131}-BSA (0.01 μg of nitrogen) bound to antibody globulin. Abscissa: log of amount of unlabeled BSA or RSA added to block the reaction (μg of nitrogen).

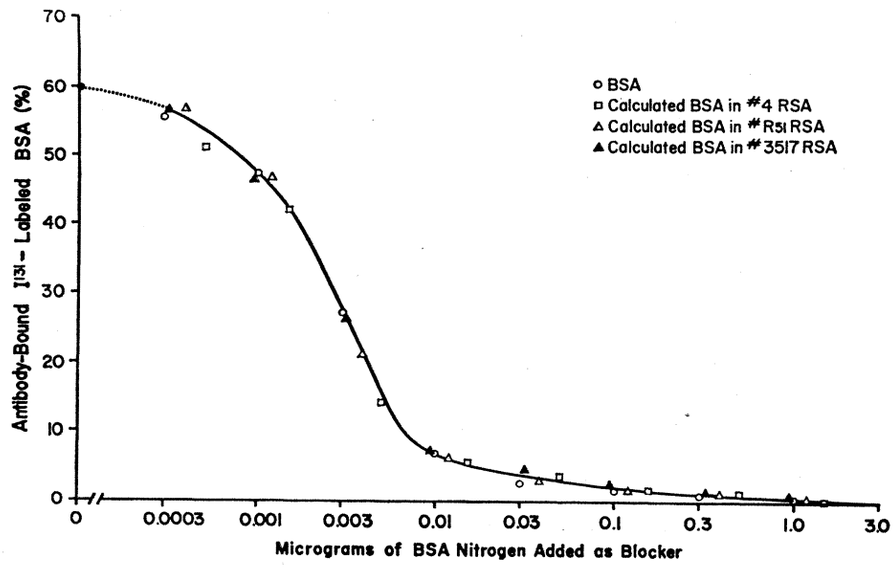

Fig. 3. Superposition of BSA and RSA blocking curves shown in Fig. 2, when corrected for BSA content of the RSA preparations. Ordinate: percent of I^{131}-BSA (0.01 μg of nitrogen) bound to antibody globulin. Abscissa: log of amount of unlabeled BSA present as blocking antigen, calculated from the amount of BSA contaminating each RSA preparation.

sufficient to bind about 60 percent of 0.01 μg (nitrogen) of I^{131}-labeled BSA. To 0.5 ml portions of this dilution of antiserum to BSA (in 10 percent normal rabbit serum) was added 0.5 ml of increasing amounts of unlabeled RSA or BSA, as a blocking antigen. After 18 hours at 0°C, 0.5 ml of buffer solution containing I^{131}-BSA (0.01 μg N) was added to each tube, followed by another 18 hours at 0°C. Then 1.5 ml of saturated ammonium sulfate was added to each tube, and the resulting precipitate was removed by centrifugation, washed, and the I^{131} content was determined. As can be seen in Fig. 2, the reaction between labeled BSA and antiserum to BSA was completely blocked by a sufficient quantity of either BSA or commercial RSA. The slopes of all the curves are quite similar, and from the amount of RSA required to produce 50 percent inhibition (0.49 to 0.75 μg of RSA nitrogen as compared with 0.00265 μg of BSA nitrogen), it could be calculated that the three RSA preparations examined contained from 0.35 to 0.54 percent BSA. When inhibition was related to the calculated amount of BSA in each increment of commercial RSA, all four curves were superimposed (Fig. 3).

The data strongly indicate that all of seven commercial RSA preparations, both crystalline and fraction V, from two different sources, contained appreciable quantities of BSA. It is most unlikely that the denaturation processes during preparation could have altered the rabbit albumin so greatly that it would cross-react 100 percent with BSA, which appears to be the only alternative explanation of the data presented. The extent to which the commercial RSA samples might also have contained proteins from species other than the bovine was not investigated. The contamination observed could probably have resulted from inadequate cleaning of fractionating equipment, and it appears to be serious enough to warrant this note of caution (*3*).

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24 July 1963

Homograft Rejection in the Fetal Lamb: The Role of Circulating Antibody

Abstract. *Specific rejection of sterile orthotopic skin homografts by the fetal lamb* in utero *was unaccompanied by the presence of plasma cells in either the graft and its bed, or in the reactive draining lymph node. The grafts appeared not to stimulate the production of circulating immunoglobulins. The presence in the fetal circulation of rabbit anti-sheep 7S γ-globulin and anti-β_{2M}-globulin did not inhibit the normal course of homograft rejection. These data support the contention that conventional circulating antibody is not an obligatory participant in the rejection of solid-tissue homografts.*

The nature of the immunologic mechanisms responsible for the rejection of orthotopic skin homografts has not yet been fully resolved (*1*). While most investigators favor the view that graft rejection involves the mechanism of delayed hypersensitivity mediated by sensitized mononuclear cells (*2*), the obligatory participation in this process of conventional circulating antibody has proved difficult to exclude (*3*). This report describes an experimental model that may provide a suitable approach to this problem. The method takes advantage of the ability of the fetal lamb *in utero* to reject skin grafts specifically (*4*), and of the fact that in this unique environment the fetus is deficient or completely lacking in immunoglobulins (*5*). This deficiency results from the limited production of these proteins by the fetus, and also from the inability of maternal antibodies to cross the ovine placenta.

Employing a modification of the technique of Schinkel and Ferguson (*4*), orthotopic fetal skin grafts were applied to the fetal lamb *in utero* between the 120th and the 139th day of the normal 150-day gestation, without interruption of pregnancy. At appropriate times thereafter the fetuses were delivered by Caesarian section and bled from the umbilical cord for serologic studies, and the grafts and representative lymphoid tissues were collected for histopathologic examination. Ten fetal lambs were grafted for the purposes of the present study; nine of these proved to be useful in that a primary take of the graft was evident. Initial healing and subsequent graft rejection could be evaluated in the usual manner. The typical pattern of epithelial junction and proliferation and vascular anastomosis was followed by mononuclear infiltration, vascular stasis, hemorrhage, epithelial death, polymorphonuclear invasion, and ultimately by necrosis and hyalinization of donor collagen. Autograft survival served as a further control of the technique.

The ability of the homograft to stimulate plasma cell and immunoglobulin formation in the fetus was studied by employing fetal skin as donor material. Full-thickness skin was taken from an aseptically delivered fetus immediately prior to use, care being taken to maintain strict surgical technique and to avoid the introduction of other exogenous antigens. The fetuses were delivered 7, 10, and 14 days later. Histologically, each of the grafts was found to have taken well, and all were in the process of typical rejection. In no instance were mature or immature plasma cells found either in the infiltrate in the graft or graft bed, or in the lymph node draining the graft site. Pyroninophilia was limited to large lymphocytes in the reactive areas of the draining lymph node. The sera of these animals revealed no detectable change from the normal electrophoretic pattern, in that the β_{2M}-globulin did not appear to be increased above the normally observed amount, and the typical 7S γ-globulin and β_{2A}-globulin arcs were still lacking (*5*).

These data suggest that the fetal lamb, demonstrably competent to form antibodies against a number of different antigens (*6*), did not respond to a sterile skin homograft with the formation either of those cells normally implicated in the production or those proteins involved in the action of circulating antibody.

In order to test more critically the suggestion in the foregoing data that circulating antibody plays no role in skin homograft rejection in the fetal lamb, four additional fetuses were grafted. In this experiment, however, the fetuses (weighing some 1500 to 2000 g) were injected intraperitoneally or intracardially with 10 ml each of rabbit anti-sheep 7S γ-globulin and rabbit anti-sheep β_{2M}-globulin at the time of the grafting procedure. In addition, two fetuses were similarly grafted, but injected with 20 ml of normal rabbit serum as an experimental control.