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Antigenic Properties of Living Tissue Cells.

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Immune serum against the cells in a tissue culture inhibits their growth completely without necessarily killing them, whereas immune sera against the culture medium, plasma and embryonal extract have no influence on the growth of the cells (Lambert and Haines,[1] Hadda and Rosenthal,[2] Kimura,[3, 4] Craciun and Sorescu[5]). These findings open a new way for investigation into certain biological aspects of the cell that would be accessible by other means only with difficulty.

On immunization with such a complicated structure as a living cell we have to assume that several different antibodies are formed against the different chemical structures. The conditions become even more complicated when we are working not with a pure cellular material but with some crushed tissue containing not only various kinds of cells, but also connective-tissue elements, blood, supporting tissue of various kinds, etc. So the fact that an immune serum, for instance, against minced embryonal material inhibits the growth of homologous fibroblasts *in vitro* gives no exact information about the components taking an active part in the process.

Then the question is: What components of the cells are so vitally important that their blocking by antibodies is incompatible with the growth and proliferation of the cell? Or, in other words: What cellular antigens produce the growth-inhibiting antibodies?

Studies on the properties of the antibody, on the other hand, appear to be of no particular interest. *A priori* it is not reasonable to expect that its nature would differ essentially from that of other know antibodies.

In searching for the active antigens, two procedures are available. One is to treat a fairly large amount of cellular material (*e. g.*, minced embryonal material, "brei") in various ways and then use the purified product for immunization. This procedure has many drawbacks.

1 Lambert, R. A., and Haines, Fr. M., *J. Exp. Med.*, 1911, **14**, 453.

2 Hadda, S., and Rosenthal, F., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1913, **16**, 524.

3 Kimura, R., *Z. f. Immunitätsforsch. u. exp. Therap.*, 1928, **55**, 501.

4 Kimura, R., *Mikrobiologische und immunol. Forsch. unter Anwendung der Gewebezüchtung*, Kyoto, Isseido, 1932, pp. 47-55.

5 Craciun, E., and Soresеu, *Compt. rend. Soc. de biol.*, 1931, **106**, 761.

For one thing, it requires a rather large amount of material that often is difficult to obtain. Several animals have to be immunized each time, as the animals differ greatly in their reaction to the injected substance. Further, this procedure takes a long time. Besides, and this is even more unfortunate, it gives no information as to the antigenic content of the different products, as the reactive power of the animals is unknown. Last, not least, if there be several different "active" antigens, they cannot be differentiated.

Therefore, the other procedure has been adopted here. The immunization is carried out with untreated cellular material (minced chicken embryo). Then the active serum is absorbed by the product that is to be tested. This procedure has several advantages. It requires but small amounts of material, a few drops or a few centigrams. The absorption takes only a short time. It gives a survey of the active-antigen content of the various preparations, as they may be used for absorption of antibody from the same immune serum (with the same antibody-content), and hence it allows of control series in both directions. If there be several different antigens, they can be differentiated through repeated absorption.

The experiments are carried out in cover-slip cultures with the following medium: 1 drop of chicken plasma, 1 drop of embryonal extract and 1 drop of the serum under analysis. The cultures are made either with fresh heart fragments from a chicken embryo or with older cultures. The latter way offers certain advantages, but it was employed only to a minor extent because it requires a great deal of work to obtain older cultures for all the large experimental series. The size of the heart fragments is an important point. As a rule, complete inhibition of growth from the very small fragments requires but a smaller antibody-content. The fragments must not be loose or frayed; if so, the growth will vary greatly within the same series.

With this procedure the active immune serum obtained by immunization of rabbits with minced chicken embryo inhibits completely the growth of cells in culture. In order to be suitable for absorption it is rather essential that the serum can stand dilution at least to 1:2 (final dilution in the culture 1:6). After being treated in various ways the capacity of the minced embryo for absorption of the active antibody is tried out and then the serum is tested in the cultures. When the absorbed serum is unable to inhibit growth, or when the inhibitory effect is lowered considerably, the conclusion is drawn that the product employed for the absorption has contained unchanged antigen.

It is found that minced embryo after washing with large amounts of physiological salt solution, and long after the biuret reaction in

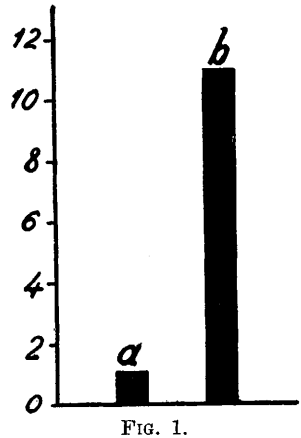

FIG. 1.

Column *a*: Active immune serum, untreated.
Column *b*: Same serum absorbed with washed minced embryo.
This experiment was carried out with cultures in the 6th passage.
The cultures are measured by planimetry at the explantation and after 4 days' growth. The square root of the initial area is subtracted from the square root of the final area, and the difference is recorded on the graph.
Each column presents the average of 5 cultures.

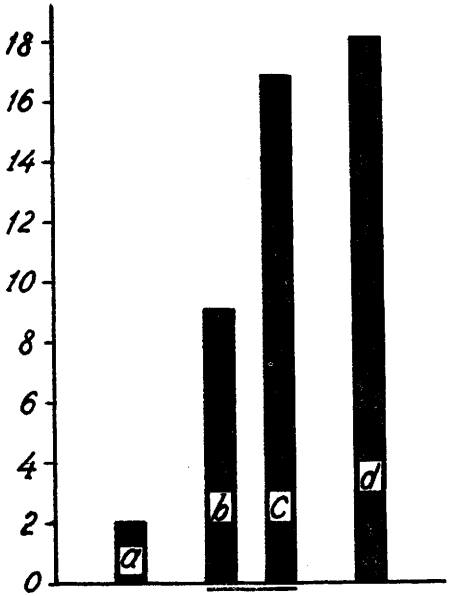

FIG. 2.

Column *a*: Active immune serum, untreated.
Column *b*: Same serum absorbed with hemolyzed erythrocytes.
Column *c*: Normal rabbit serum absorbed in the same manner.
Column *d*: Normal rabbit serum, untreated.
This experiment was carried out with cultures in the 10th passage. Each culture is divided in 4 pieces, one piece for each series. Each column presents the average of 5 cultures.
Measuring and calculation as in Fig. 1.

the wash water has become negative, still is able to absorb the antibody (Fig. 1). Likewise, after standing for a considerable length of time (10-15 days) and repeated washing with physiological salt solution, minced embryo is still capable of antibody-absorption. The same applies to hemolyzed erythrocytes. When employed for immunization the erythrocytes give rise to production of growth-inhibiting antibodies (Kimura[4]), containing thus the active antigen.

I have not been able to obtain any measurable absorption of the growth-inhibiting substance on intact erythrocytes in the proportion of mixture I have employed (1 part 100% erythrocytes + 1 part serum). If, on the other hand, the erythrocytes before this procedure are hemolysed in a hypotonic salt solution, they regularly absorb the antibody in the same proportion of mixture. This prelim-

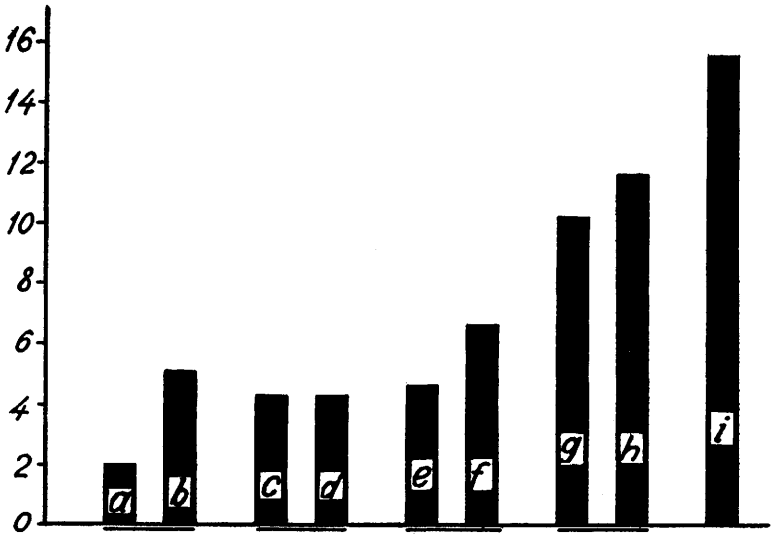

FIG. 3.

Column *a*: Active immune serum, untreated.
Column *b*: Active immune serum, diluted with an equal amount of Ringer's solution.
Column *c*: Active immune serum, absorbed with *washed* minced embryo, 5 drops embryo to 25 drops serum.
Column *d*: Active immune serum, absorbed with *unwashed* minced embryo, 5 drops embryo to 25 drops serum.
Column *e*: Active immune serum, absorbed with *washed* minced embryo, 10 drops embryo to 25 drops serum.
Column *f*: Active immune serum, absorbed with *unwashed* minced embryo, 10 drops embryo to 25 drops serum.
Column *g*: Active immune serum, absorbed with *washed* minced embryo, 25 drops embryo to 25 drops serum.
Column *h*: Active immune serum, absorbed with *unwashed* minced embryo, 25 drops embryo to 25 drops serum.
Column *i*: Normal rabbit serum.
Measuring and calculation as in Fig. 1.

inary hemolysis has to be carried out very gently, however, in order to avoid the formation of clear gelatin-like lumps (nucleic acid?). Serum absorbed on hemolysed erythrocytes shows only a slightly greater fall in hemolytic titer than does serum absorbed on the same amount of intact erythrocytes, but it loses a considerable part of its growth-inhibiting effect.

It seems rather reasonable therefore to think that the interior of the erythrocytes contains the active antigen, at any rate in a larger amount than the surface of the cell, and that this antigen is not identical with the one that gives rise to the formation of hemolysin. But this question requires further investigation.

Minced embryo appears to lose some of its absorbing effect by being washed (Fig. 3).

No evidence was found to suggest that the antigens removed by absorption are qualitatively different from those that remain. Otherwise it is one of the most important questions of the whole problem, whether distinction might be made between different antigens through serial absorption of the same portion of serum by different purified products.

It appears as if washed minced embryo relatively soon loses a considerable part of its absorptive power when it is kept in the icebox. Unfortunately, I have not had time to look into this question systematically. Here, among other things, it is important to ascertain whether the decrease in absorptive power be due to a loss of certain definite antigens or merely a quantitative falling off. Minced embryo triturated with kieselguhr and washed afterwards retains all its absorbing effect. This, together with the experiences with hemolysed erythrocytes, indicates that the "unsoluble" submicroscopic stroma of the cell constitutes the active antigen. If the stroma were a sort of stable inert framework in the cell, one would hardly expect its blocking by antibody to have such a striking effect. Also the lability of the antigen, as manifest on storage of the washed minced embryo, suggests that the stroma is to be looked upon as a functional structure capable of reaction. Here it is to be mentioned, however, that even though the active antibody cannot be absorbed on the surface of the red blood cells, it is by no means established that the inhibition of the growth of the fibroblasts may not be associated with the surface of the fibroblasts. The relation between the amount of intact or living cells in the minced embryo employed for the absorption and its capacity for absorption requires further investigation. By heating at 56° for 30 min. the minced embryo loses its absorptive power. But the immune serum can stand this heating without losing any of its inhibitory effect. Thus complement is not necessary for the inhibition

(Kimura[4]). In undergoing autolysis, by being placed in the incubator at 39° for 5 days, minced embryo loses its absorptive power.

Dr. Albert Fischer has observed that when two tissue cultures are excised and transferred to a tube containing physiological salt solution, they coalesce in their growth, *i. e.,* they grow into each other, and of the two cultures there finally remains only one small spherical block of tissue.

It is found that the anticellular immune serum is able to prevent this coalescence. This observation appears to imply a new way of reaction by which the antibody in the growth-inhibiting immune serum may be titrated. Here it involves a sort of "reverse agglutination." Also fragments of heart may be employed for this reaction, but then the heart has to be incubated previously for 2 hours, or left standing in the refrigerator overnight, as otherwise lymph will exude from the small fragments, and coagulate, preventing thus a close apposition of the fragments. The reaction is carried out in small tubes with a slight diverticulum of the bottom where the fragments are placed so that they can be in contact with each other. The layer of fluid above the fragments should not be too high (0.5 cm), and it is advisable to add a little serum and embryonal extract to the fluid. The size of the fragments plays some rôle too.

Owing to external circumstances my studies had to be discontinued at the present stage. Therefore, I find it appropriate now to make my experiences available to others.

I am greatly indebted to Dr. Albert Fischer for his valuable criticism of my studies as they were progressing.

The work here reported has been carried out by means of support from the Legacy of Consul General Ernst Carlsen and his wife.