Resumen por el autor, R. S. Cunningham.

La reacción de las células que tapizan la cavidad peritoneal,
incluso el epitelio germinal del ovario, a los
colorantes vitales.

El autor ha observado que las células de la serosa que tapiza la cavidad peritoneal almacenan los colorantes vitales de una manera muy característica. Las dos manifestaciones más sorprendentes de esta reacción consisten en la localización de la concentración de los gránulos tintóreos en un área circumsrita del citoplasma de cada célula y en la formación de una roseta perinuclear. Las células mesoteliales de diferentes áreas de la superficie peritoneal presentan ciertas particularidades en sus reacciones con los colorantes vitales, las cuales son suficientes para clasificarlas en grupos mientras que todavía se conforman con el tipo general característico. Las variaciones notadas en las células de diferentes áreas de la superficie peritoneal consisten en diferencias en la cantidad de colorante almacenado, las características de la roseta perinuclear y la orientación de la acumulación localizada de partículas tintóreas dentro de la célula. Las células que cubren al intestino contienen generalmente la menor cantidad del colorante, mientras que las del mesotelio esplénico y las del epitelio germinal del ovario contienen la mayor cantidad. El epitelio germinal almacena los colorantes vitales de una manera especial y característica. Cada célula contiene una masa de gránulos, redonda, oval o en forma de copa, en la zona infranuclear de la célula; esta masa, en los ejemplares bien teñidos, llenaba toda la porción de la célula situada entre el núcleo y la membrana basal. Por otra parte, la roseta perinuclear ha sido hallada solo raras veces en las células del epitelio germinal.

THE REACTION OF THE CELLS LINING THE PERITONEAL CAVITY, INCLUDING THE GERMINAL EPITHELIUM OF THE OVARY, TO VITAL DYES

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ONE PLATE (EIGHT FIGURES)

The general trend of the large amount of work carried out on the nature and significance of vital staining has been to indicate a definite relationship between all the cells which manifest a reaction to these dyes in the same way and to the same degree. That many observers have been extravagant in regard to the various applications of this theory may be admitted, while the strict value of the principle is still adhered to. Again it has been customary to consider as important only those reactions to this group of dyes in which large, or at least moderate amounts were stored. All cells having a smaller content were more or less huddled together and left unstudied. Yet the careful study of the characteristics in those groups of cells where only minor amounts of vital dyes are stored may prove of as much interest and importance as the elaborate study of those cells in which the power to store these substances is very great. This is, I think, further suggested in the recent brilliant studies of Evans and Scott ('20) on the reactions of clasmatoocytes and fibroblasts. This work, though directed towards the differentiation of these two cell-types in as exact a way as possible, revealed much of the reaction of the fibroblasts with different exposures to many vital dyes, and it is practically certain that these observations will later be of very great value in increasing our knowledge of the phases of fibroblastic activity.

Since the cells lining the peritoneal cavity do not store vital dyes in large amounts, they have been among those to which but little attention has been paid. But the finding that the

reaction of these cells is most specific in character, even though the amount of dye stored is much less than in the case of the clasmatocyte, has suggested that this may be a method which will assist in solving some of the long-discussed questions regarding the relationships which the cells lining different areas of the peritoneal cavity bear to each other and to other cells.

Goldmann, in his studies on vital staining, gives very brief consideration to the peritoneal lining cells. In one place ('12, p. 40) he states specifically that the 'endothelial serosal lining cells' do not store any vital dyes, and in another ('09, p. 45) he records the observation that the germinal epithelium of the ovary likewise does not stain in the living animal.

Pappenheim ('13) and Pappenheim and Fukushi ('14) agree with Goldmann that the peritoneal mesothelial cells do not take vital stains. They use this lack of the ability to store vital dyes as an argument against the participation of the lining cells in the formation of the free phagocytic cells of inflammatory exudates. Tschaschin ('13) carried out experiments using collargol, isamine blue, and trypan blue, and states that he found that the serosal lining cells remained entirely unstained.

That peritoneal mesothelium stains to some extent, however, when exposed to vital dyes has been noted by Schlecht, Evans, Kiyono, and Foot. Kiyono ('14) has given the most elaborate description of the vital staining of mesothelium, and his are the only illustrations of vitally stained serosal lining cells which I have been able to find. His figure (a), plate (1), shows two cells containing dye from animals which had been vitally stained by intravenous injections of carmine; these cells he designates as peritoneal lining cells, but he does not state from which surface they were obtained. Kiyono also studied the reaction of these cells to trypan blue in the omentum and found that the distribution of the blue was precisely the same as that found for carmine. He suggests that the reason the trypan blue granules have not always been found in the mesothelial cells is due to too long fixation, during which the blue diffuses out of the surface cells. He fixed his omental spread preparations for one hour and then studied them for the distribution of the dye. He

describes a concentration of the dye-granules near the nucleus, but suggests that this is due to the greater amount of cytoplasm in that region, and he intimates that there are as many granules elsewhere in relation to the amount of cytoplasm. The granules, which he found, were always small and evenly distributed without accumulations in any particular areas, or irregular clumping as has been found in the clasmatocyte. His plate (1) gives a most interesting comparison between the immense amount of carmine which has been stored by the clasmatocytes and monocytes, and the sparse granulation of the serosal cells. On the other hand, the fibroblasts show amounts of carmine not very different either in amount or distribution from that of the mesothelium. Kiyono thinks that his work with vital dyes indicates a very definite and close relationship between the serosal cells and the fibroblasts, though he finds no actual transition between the two cell-types.

Evans ('15) has also described the sparse granulation of the serosal cells, noting that the fine granules of dye were concentrated around the nucleus, but his descriptions are very brief and do not indicate whether or not he agrees with Kiyono in regard to the more general distribution of dye-granules throughout the cytoplasm.

Schlecht ('07) also describes the peritoneal mesothelium as taking vital dyes, but does not differentiate very sharply between the surface-cells and connective-tissue cells, which probably also include some true clasmatocytes. His observations are not very exact with regard to the serosal lining cells, but are sufficiently so to indicate that there are some vital dye-granules to be found in the serosal mesothelium.

Tschaschin, on the basis of his studies with vital dyes ('13 a) and on experimental inflammations ('13 b), has concluded that the serosal lining cells constitute a specific group, having no relationship with the connective-tissue elements.

Evans and Scott ('20, p. 47) have referred to the subject of the specificity of mesothelial cells as distinct from fibroblasts and clasmatocytes in their monograph on the connective-tissue cells. They agree entirely with Tschaschin in considering the

lining cells as a distinct strain and state that the use of the azo-dyestuffs has furnished, in part at least, a means of differentiating these cells. They do not give any very specific discussion of the characteristics which the surface-cells display other than that the granules are always minute and that the spleen manifests them in the most marked degree. That they have observed many of the characteristic differences which the cells lining the peritoneum manifest towards vital dyes is undoubted, and their opinion is clearly in favor of their specificity, but the analysis is insufficient for establishing any basis regarding the especial peculiarities of these cells. Undoubtedly an examination of these cells with a large number of dyes would yield further interesting facts.

Ribbert ('04), Schlecht ('07), Goldmann ('09), Kiyono ('14), and Evans ('16 a, '16 b) have described the vital staining of the atretic follicle of the ovary. But none of these authors describes any staining of the germinal epithelium, Goldmann alone referring specifically to these cells, stating that they do not store any vital dye at all.

Foot ('19, p. 366) reports the observation that the serosal cells from the omentum stain deeply with vital dyes, and uses this reaction to suggest their having a genetic relationship to free cells formed during inflammatory reactions. In a later communication ('21, p. 635) he describes the serosal lining cells of the omentum as containing a few fine granules, and concludes that most of the dye appears to pass through the cells.

Most of the work that has been done on the cells lining the peritoneal cavity has been directed to the solution of the problem of relationship between these cells and the free cells of inflammatory exudates. But the serosal lining cells are of interest in other connections, among these the most important are their relationship to fibroblasts and to the fundamental physiological activities in which the surface-cells are adapted to mediate. The phenomena of vital staining is of very great importance in differentiating cell-groups from cell-groups and has already assisted very much in outlining the absolute separation of serosal cells from clasmatoocytes. Can it also be used to assist in establishing

the relationship which exists between the fibroblast and the serosal cell? Finally, a careful study of the way in which serosal lining cells react to vital dyes may throw some additional light on the interrelationships of different areas of mesothelium and on the physiology of these cells, both individually and as a living membrane.

MATERIAL AND METHODS

It is obvious at once to anyone who has studied the cells lining the peritoneal membrane that sections cut in the ordinary manner perpendicular to the flat surface of the cells could yield but little information as to the extent or arrangement of the dye concretions in the entire cell. It is, I think, this fact that has caused so many observers to conclude that the cells are entirely free from the dye; while most of those who have described the granulation have studied the cells in the omentum where they can be observed in their entirety by means of spread preparations.

The observations reported here have been made from a series of experiments on rabbits. The animals were stained with Grüber's trypan blue or with carmine prepared according to Kiyono's method. Both dyes were usually administered intravenously at intervals of twenty-four or forty-eight hours, the animals received from six to thirty doses of carmine, from three to twenty doses of trypan blue, and were usually sacrificed one to two days after the last injection.

In the study of the cells several methods were used. The animal was anesthetized and the abdomen opened, cells were scraped from the surfaces of all the viscera, diaphragm, and body-wall, and were studied immediately. Smears were also prepared in a similar way, fixed by heat or alcohol and then stained in an appropriate manner to obtain good contrasts. Spread preparations of omentum and mesentery were studied fresh. Omental spreads were fixed by immersion for one hour in osmic acid or neutral formalin, washed quickly and stained; others were treated with weak ($\frac{1}{4}$ to 1 per cent) silver nitrate, exposed to sunlight, and then fixed in alcohol. Very thin blocks of all the organs which border the peritoneal cavity were fixed in neutral formalin for one hour, and then transferred to 80 per

cent alcohol, dehydrated, and embedded as rapidly as possible. Blocks were also fixed in 95 per cent alcohol, it having been found that the dye did not diffuse out to any appreciable extent from tissue fixed in strong alcohol. Sections were cut parallel to the surface and all the surface sections carefully preserved in series. In this way some slight shrinkage of the tissues occurred as determined by control preparations, but any loss of dye from the cells was reduced to a minimum. Finally, very thin membranes were obtained by stripping small bits of the surfaces of organs while the blocks of tissue were in 80 per cent alcohol; these were stained and cleared, and while many were entirely too thick for careful study, others were obtained which were as thin and easily analyzed as omental spreads. In general there seems to have been some loss even in these preparations, as could be determined by the control studies in fresh material, but the loss was in no way sufficient to prevent cellular orientation, which is obviously the one difficulty in the interpretation of the cells removed by scraping.

It is a pleasure to thank Mr. Didusch for the care and accuracy with which he made the excellent drawings.

EXPERIMENTAL RESULTS

The cells which line the peritoneal cavity may be divided into four classes by means of the reactions to vital dyes. These are all closely related to each other, agreeing in certain particulars which seem to be very fundamental, while they differ in minor points which are probably in some way related to the function of the organs which they cover. The classification into four groups is not to be considered as absolute, because variations in one may approach the normal in another, but the general types are sufficiently distinct to require separation.

1. General serosal mesothelium, including the cells covering:
 - a.* intestine.
 - b.* body-wall.
 - c.* liver, pancreas, etc.
 - d.* mesentery.
 - e.* diaphragm.

2. Cells covering the omentum.
3. Cells covering the spleen.
4. Cells covering the ovary, the so-called germinal epithelium.

The first division is a composite one, including all the cells which have no very sharp differences from each other, and besides all of these cells agree in general in storing smaller amounts of the dyes than do those of the spleen and ovary and differ from the cells of the omentum in certain peculiarities other than the amount of dye stored. The lining cells of the mesentery are very much like those of the omentum, but have certain local differences which are more like the general lining cells elsewhere, thus placing the cells covering the mesentery midway between the ordinary serosal lining cells and the cells covering the omentum. In this way there are all gradations between the cells covering the intestine which take less vital dye than any other serosal cells, and the cells covering the spleen and the ovary, both of which show a characteristic vital staining with considerable ease. The other variations and what evidence there is of a parallel physiological relationship will be taken up later.

The most obvious characteristic which has been observed in the manner in which the lining cells store vital dyes is the collection of the dye concretions into an area more or less regular in shape in a definite portion of the cytoplasm. The distribution of dye-granules in a perinuclear rosette is also characteristic of the serosal cells, but requires longer exposure to the dye for its development and is never so universally present as the specific separate mass in the cytoplasm. These two specific types of staining are often found combined, but each and particularly the clump of granules may appear alone or with many minor variations, which are in part characteristic of the cellular surface, in part the result of the kind of dye employed, but to a greater extent due to the length of time during which the cells have been exposed to the dyes. Again it has been noted in general that carmine tends to remain more definitely collected in a small area, while trypan blue tends much more to form perinuclear rosettes, usually with, but sometimes without, local concentrations. It

is quite likely that still other variations than those described would become apparent if a larger series of dyes could be employed, and they might prove of value in further subdividing the serosal cells into smaller groups.

General serosal mesothelium

In animals acutely stained with trypan blue (i.e., having received six to eight doses), the cells covering the intestine, the body-wall, the liver, the mesentery, and the diaphragm had in common the distribution of dye-granules in a fine irregular, perinuclear rosette with a more or less evident concentration at some area in the cytoplasm. There were a few scattered granules besides, usually just adjacent to the small clump. In animals having had only three to five doses, the perinuclear rosette was usually represented by an occasional granule and the small cytoplasmic clump at this earlier stage was present as a small irregular ring of granules. This ring was usually the first evidence of dye in the cell and often formed an almost perfect ring or oval, usually about one-half or one-third the size of the nucleus. The most conspicuous individual differences between the individual surfaces included in this group, as shown in acute staining with trypan blue, were the greater number of diffused granules in the diaphragmatic cells, the more sharply formed ring in the mesentery, the greater tendency to perinuclear rosettes in the liver, and the generally sharper localization and less amount of dye in the body-wall and intestine. These differences are given more because they indicate the relative lack of extremely sharp differentiation than because they seem of great importance within themselves.

In animals in which staining was carried beyond eight to ten doses of trypan blue, the most important changes were a general increase in the amount of dye around the nucleus in all the cells of these surfaces except the diaphragm where there was a more definite increase in the granules diffusely scattered around the circumscribed area of earliest staining (fig. 2).

Turning now to the reactions of this group of cells to intravenously administered carmine: in the early stages when the blue was found in a ring with a few scattered perinuclear granules the carmine was almost always in a small round or oval patch, always sharply localized in the cytoplasm. These carmine granules were almost always very small, uniform in size and evenly distributed. The regularity with which the early carmine staining showed this precise arrangement was quite remarkable. When the experimental animal had received more dye, ten to fourteen injections, the principal difference noted was the increase in the size of the patches of carmine granules and the beginning of the perinuclear rosettes. In animals in which the staining was carried much further—fifteen to thirty doses—the size of the isolated groups of dye-particles increased still more, and in many cases there were some granules which had apparently strayed away from the central fold so that these areas were no longer so sharply outlined. This distribution of the dye was particularly characteristic of the cells covering the diaphragm, the irregularity of the granules almost furnishing sufficient degree of difference to permit the classification of these cells in a separate group. These diaphragmatic mesothelial cells were the first to show perinuclear rosettes, though these were often most irregular and diffuse. Figure 8 shows two cells which were obtained from the surface of the diaphragm of a rabbit which had received twenty-five doses of carmine on alternate days; both the diffuse perinuclear arrangement and the irregularly scattered cytoplasmic granules are shown, but nevertheless there is quite definite evidence of a greater concentration of the dye in a definite part of the cell. Only in very rare instances have cells been found from any of the surfaces of the general serosa that did not present this wholly characteristic type of staining.

In animals stained over a considerable length of time, surface-cells other than those of the diaphragm also contained more carmine granules, but distributed usually with greater regularity. In those over the stomach and intestine the additional granules were usually added to the preexisting small ring or mass, while

a few scattered granules sometimes developed about the nucleus. In the cells covering the liver particularly and the other surfaces to some extent, the carmine granules, with increased staining, often developed into a long, oval belt which sometimes crossed the nucleus only on one side, and sometimes extended partially if not wholly around it. So that instead of having the circumscribed perinuclear rosette which has been described as characteristic of trypan blue, these cells had a rosette in the form of a belt running all, or part way, around the nucleus antero-posteriorly.

Finally, it seems important to emphasize the question of the orientation of the dye distribution in relation to the nucleus and the position of the cells in situ. These observations were made both upon living cells and upon sections, the latter being cut perpendicular to the surface. In sections the perinuclear rosette so typical of trypan blue was clearly seen as a patch of granules at either end of the nucleus, one or the other containing more blue corresponding to the area of localization. This was often extended somewhat over the surface of the end of the nucleus. But the characteristic arrangement was the principal amount of the blue close to one end of the nucleus. In the early staining with carmine the small sharply localized area of granules was usually between the nucleus and the surface of the cell, but placed laterally to the central axis of the nucleus, so that as the dye increased in amount with longer exposures the cross-sections showed red granules in both outer and inner zones of the cell. This again was the formation of the perinuclear belt, already described, from the lateral or anterolateral group of granules. Considering the relationship of the early areas of carmine staining to the center of the nucleus as distributed along its long axis, they were found to be usually somewhat nearer one end, but were often close to the center. As the carmine increased in amount to the maximum, the granules often covered the entire length of the nucleus, but more often formed the perinuclear belt.

The omentum

When the omentum from a rabbit that had been vitally stained with trypan blue was spread on a cover-slip, treated lightly with silver nitrate and stained with carmine, the large pavement cells of the serosa were found to be very characteristically stained. They almost always contained a small ring of blue granules in one part of the cytoplasm, sometimes this ring was entirely regular and smooth in contour and again it was broken or oval shaped. These granules appeared in animals which had received only a few doses of blue; when the staining was carried further, the cytoplasm in the neighborhood of this first ring-like group of granules began to show a few additional granules, and finally the perinuclear rosette developed. In an animal in which the staining was carried out with the usual amount of dye, six to eight doses, the sharply defined area was very characteristic and the ring was still present though obscured by the development of some diffuse granules adjacent to it. The perinuclear rosette was present in about one-half to one-third of the cells, but was usually quite irregular. Perhaps the most interesting and characteristic finding was that, in the majority of the cells, the ring and its subsequently attached granules were opposite the long axis of the oval nucleus (fig. 5). Most of the mesothelial cells covering the omentum were rather elongated, and with the patches of dye-granules located in one end they presented a very characteristic appearance. With longer exposures to blue, the granules gradually extended throughout the end of the cell and a few appeared irregularly elsewhere in the cytoplasm, increasing the sharpness of the perinuclear rosette, and sometimes appearing over or under the nucleus. Occasionally there were two sharp areas of localization in a single cell, but this was not common in the normal cells except in those where there were two nuclei; in such instances the arrangement was usually of one or two types. In many cells the dye-granules were arranged about each nucleus as though the cell might be in process of division, suggesting that each group of granules eventually would be entirely characteristic of an individual cell. In others there

were granules massed between the nuclei, and this mass extended arms of dye which surrounded the two nuclei in more or less irregular rosettes. In the one case the arrangement suggests that the two nuclei are functionally the centers of separate cells, while in the other the arrangement implies that the cell is truly binucleate and functionally a single unit. The collection of granules opposite the long axis of the nucleus was not always present, a few cells in every preparation having the principal mass at the side of the oval nucleus, but the proportion was greatly in favor of the other type. A few granules were often seen scattered between the nucleus and the surface or in the infranuclear zone, but the principal mass was very seldom in either of these two regions.

In animals stained with carmine the same distribution was observed with certain modifications. The mass of carmine in the end of the cell often extended until it almost filled the entire cytoplasmic area, but always the borders were more regular than with the trypan blue, the irregular scattered granules being much less numerous. The perinuclear rosettes were more infrequent, or rather were relatively later in appearing in the case of carmine. However, in very heavily stained animals they were to be seen in many cells, long fine arms of granules extending irregularly across or around the nucleus, or perhaps out into the free areas of cytoplasm. A similar condition is shown in figure 7, which is from the spleen. It is interesting to recall that the cells of the omentum show much more diffusely scattered granules after they have been irritated than do the cells of the other areas, especially of the spleen (Cunningham, '22), but the significance of this still remains to be studied.

The spleen

In the cells covering the spleen the perinuclear rosette developed very early in the course of staining with trypan blue, but it was usually accompanied by some condensation into the type of arrangement which has already been described for the general mesothelium. As has been noted, this blue rosette

surrounded the nucleus parallel to its flattened surface, so that the condensation did not, as a rule, develop between the flattened surface of the nucleus and the surface of the cytoplasm, but rather it was adjacent to some point of the periphery of the nucleus. The numerous apparently different arrangements are all easily comprehensible if this fundamental type of the distribution of the dye is clearly understood. Despite the fact that in surface cells of the spleen the rosettes developed early and quickly, they contained only a small amount of dye before the condensation of granules became the most obvious part of the staining. These collections of dye very rapidly increased in amount until in smear preparations the cells often seemed to contain only this single large mass of dye. The location of the mass of dye in relation to the nucleus was very interesting, and while every conceivable appearance was found both in smears and sections, careful study revealed that the dye-masses were, in the vast majority of cells, located lateral to the flat surface of the nucleus and near the termination of its shortest diameter in the periphery. The rapid increase in the mass produced the type of cap seen in figure 4, this cell having been selected because the perinuclear rosette was represented by only a few granules, while the entire lateral surface of the nucleus was covered by the dye. In animals having received about eight to ten injections of trypan blue, this type of arrangement was very common, though more granules were usually present in the perinuclear rosette; the cells shown in figures 3 and 4 were both from an animal in this stage of staining. In sections from the spleen at this stage the nuclei of the mesothelial cells were bordered at either end, the 'ends' being obviously the optical appearance—rather than the anteroposterior poles—of the long axis of the nucleus, by groups of blue granules varying in amount from a small number to a large patch, practically filling the cytoplasm adjacent to the nucleus. The amount of the dye in such sections was obviously dependent upon where the section divided the cell in relation to the principal mass of dye.

On further increasing the amount of dye administered, the edges of the principal dye-mass extended irregularly further and

further around the nucleus, until, in many cases, in sections, granules of dye were found in either or both of the supranuclear and infranuclear zones of the cells. With such increased amounts of dye administered, the cell-body increased considerably in thickness usually throughout the entire length, though a supranuclear bulge was occasionally present. This arrangement was constantly found in most of the cells, but there was a small proportion in which certain characteristic variations appeared. In some cells the location of the primary concentration was between the center of the nucleus and the pole of the long axis; in such cells the extension of the mass led to considerable irregularities in the relation between the nucleus and its cap of blue. Some of these caps extended around or partly around the pole of the nucleus in an irregular fashion. Again, a few cells were noted in which two localized aggregations were situated on opposite sides connected by arms of blue extending around the nucleus from one to the other. These double caps were found on the two poles as well as on the two flattened sides of the nucleus, although they were more numerous in the latter location. Again, it was found that the lateral mass had extended almost entirely into the infranuclear zone and had produced a reaction somewhat similar to that seen in the germinal epithelium of the ovary. Finally, it must be noted that the lateral location of the dye-mass, extending a fine outline of granules around the nucleus to form the typical perinuclear rosette, may, I think, be considered as the one most striking characteristic of the splenic mesothelial cells of the rabbit when vitally stained with trypan blue.

Turning now to the results obtained with the intravenous administration of carmine, the cells of the splenic mesothelium were found to be equally as characteristic as when stained with trypan blue. In the early stages the carmine was found distributed in the usual sharply localized area just lateral to the center of the nucleus and always superficial to it. As the amount of dye was increased, the area extended more and more on all sides, sometimes in a regular fashion, sometimes with fine lines and a few detached granules; figures 6 and 7 illustrate two stages in this progressive increase. The perinuclear rosette was some-

times found at about this time, but was always most irregular and never so sharp and characteristic as seen with trypan blue. The area of the cell just over the nucleus, which contained the localized mass of dye, was humped up often to a considerable extent. When the staining was carried to the maximum used in these experiments, the edges of the supranuclear mass extended irregularly around the nucleus and reached the infranuclear zone, in this way forming a perinuclear belt, similar to, but much more extensive than, those found in the cells of the general serosal mesothelium. The cells had by this stage increased considerably in size and over a larger surface than at the stage when there was only a supranuclear swelling. In some few cells the mass of dye seemed to be around the pole of the nucleus, probably the first-formed area was somewhat displaced from the region of the center of the cell. This cap formation around the longitudinal pole of the nucleus was not as common as with trypan blue. The principal difference observed in the reactions of the cells to the two types of dyes was the greater tendency of the carmine to enter the supranuclear zone and to remain circumscribed, while the trypan blue extended towards the periphery and usually towards the infranuclear zone. In general the cells covering the spleen contained considerably more dye than any other serosal cells at the same general stage of staining.

It has been reported elsewhere (Cunningham, '21, '22) that, in cats in which the splenic mesothelial cells had been irritated, the trypan blue granules tended to collect in the infranuclear zone of the cell together with many highly refractive droplets which were developed during the progress of the irritation. Further, it was found that in experimental hydremia in cats the fluid always collected in the infranuclear zone. The normal vital staining in the cat likewise tends towards the perinuclear rosette, but with a considerably greater tendency to the infranuclear extension. Whether these differences are very important cannot be stated as yet, sufficient data on long-continued irritations of the serosa of heavily stained rabbits not being completed. The area which shows the most active vital staining

probably is important in relation to other activities, or potential activities, of these cells. No further conclusions can legitimately be drawn from these observations at the present time.

The ovary

The cells covering the ovarian ligaments in the rabbit are the ordinary, normal, flat mesothelial elements, but at the point of attachment these cells pass by rather an abrupt transition into the cuboidal cells which constitute the investiture of the ovary proper. These cells, commonly known as the germinal epithelium of the ovary, are cuboidal in shape and have clearly defined, sharply staining nuclei. They seem to rest upon a very definite basement membrane and the nuclei are placed in general about midway between the surface and the base.

When vitally stained these cells stored the dye in the infranuclear zone, but only in relatively small amounts in comparison with the amounts which were taken up at the same time by the elasmatocytes and other cells commonly known as the more active in their reaction to the vital dyes; so that in lightly stained rabbits there were very few granules to be seen in these cells, not more than were found in the cells of the peritoneum generally. An increase in the amount of stain given caused a greater increase in the amount of dye which was stored by the cells covering the ovary, and a very prolonged course of staining produced an astonishing picture; the amount of dye in the cells increased very greatly until the bases of the whole layer seemed entirely filled with the dye (fig. 1). A more accurate description of the vitally stained germinal epithelial cells is given from one experiment that demonstrates especially well the very large amount of dye that can be stored: the cells of the germinal epithelium varied considerably in size and shape, usually they were cuboidal with a tendency to columnar form, the outer border occasionally tended to be rounded and to show slight indentations between the cells. The nuclei were relatively clear and stained well. They were located near the center of the cell, but there was a slightly wider zone between the nucleus and the basement

membrane than between the nucleus and the surface. Here and there a cell contained a vacuole in the base of the cell, though it was very difficult to determine whether these were inter- or intracellular. Most of the cells had no vacuoles. But every cell, without exception, contained a group of trypan blue granules near the basal pole of the nucleus and often forming a slight cup around it. Careful examination showed that there were a considerable number of types of arrangement; some cells contained an irregular circle of dye-granules; others a solid, round clump; others, a cap on the lower pole of the nucleus, and some, an irregular patch with knobs, projections, etc. Here and there an occasional granule appeared in the upper part of the cell, or a line of them extended up around the nucleus in an arm-like manner. Some of the cells were very heavily laden with granules which extended often up around half of the nucleus. Practically all the blue granules were the same size, about $\frac{1}{4}$ to $\frac{1}{2} \mu$ in diameter. None of them was very large, as in clasmotocytes, and yet few were very fine, as in ordinary normal mesothelial cells. Here and there one found cells which were considerably flatter, being about half cuboidal, and here the dye-granules were again located in the region of the cell just medial to the nucleus. These flattened cells seemed to be from the edge of the ovary near its attachment and suggested a transition to normal peritoneal mesothelium. When the transition from the flat mesothelium to the cuboidal germinal epithelium was studied it was found to be most interesting. Here transition cells showed less and less staining in the characteristic area down to the ordinary mesothelial cell with its patch of granules and perinuclear rosette.

Animals stained with carmine presented precisely similar pictures, the variations from the reaction to trypan blue were very considerably less than in areas such as the spleen or diaphragm. But there was slight tendency to surround the nucleus, and the irregularities of staining were always more sharply shown in the animals stained in trypan blue than in those stained in carmine. In many areas in the sections of the ovaries from animals stained with carmine, cells were found just beneath the layer of the germinal epithelium proper and forming a nest-like

mass bordered beneath by the basement membrane. These cells were stained exactly like the cells of the surface, having a small patch of the red granules around one pole of the nucleus, though they were not oriented in any especial manner. Whether these cells are derivatives of the layer of germinal epithelium or whether they are other cells reacting in a similar manner to the dyes is not yet clear.

DISCUSSION

The characteristic reaction to vital dyes which has been described for the general peritoneal mesothelium certainly suggests that there is a similarity in function which extends throughout the membrane, and perhaps may even include the germinal epithelium of the ovary. The differences noted between diverse areas such as spleen and diaphragm may eventually prove to be specifically bound up in the peculiar functions of the organs which they cover, or may be due to differences in environment, such as blood supply, movements of viscera, or other physical factors. The characteristic location of the vital dye-granules in the various types of mesothelial cells may have some bearing on the differences in the physiological activities of the organs which the cells cover, but our information is far too limited at present to even hazard a guess concerning these interpretations.

Obviously, the observations reported here may assist in the settlement of two general questions. In the first place, there has been much discussion regarding the relationship between the fibroblasts and serosal lining cells, some authors having maintained them to be the same fundamental type of cell manifesting different physical characteristics under different environments, and others having considered them as entirely different final types. The second question, in regard to which these findings may be of importance, is the relationship between the germinal epithelium and the general serosal mesothelium, on the one hand, and between the germinal epithelium and the structures of the ovary proper, on the other.

Evans and Scott ('20) have succeeded in separating the fibroblasts from the clasmatoocytes by their reactions to vital dyes,

but they have done even more in giving us a very large number of figures of fibroblasts stained with various dyes. In studying their plates it becomes evident that there is no pattern which one can consider as in common between even a small number of the fibroblasts.

The typical fibroblast therefore shows no characteristic staining in a definite area of the cytoplasm such as is so evident in the serosal cells from the entire peritoneal membrane. Indeed, the reactions of the fibroblast to vital dyes would seem from these plates alone to ally them more closely to the clasmatocytes than to the serosal cells. Many observers have described reactions of connective-tissue cells and serosal cells as being quite similar during the different stages of inflammations, and on such grounds as these have considered that they are interchangeable (Cornil, '97; Ranvier, '93; Schott, '09; Weidenreich, '07; Roloff, '94, '96, and Dominici, '01, '02).

Clarke ('16), who introduced celloidin and paraffin into the general connective tissues, considered that he had produced a true experimental mesothelium which, according to him, was entirely comparable to the lining cells of the peritoneum. Among the older workers many thought that the mesothelial cells were capable of transformation into a 'variety of forms,' one of which was the fibroblast. Schott ('09) and Weidenreich ('07) suggest that the surface-cells of the omentum are merely flattened fibroblasts. Mallory ('20), in studying tumors of the arachnoid, has concluded that they are of connective-tissue character, and because of these tumors arising from the arachnoidal cells, he concluded that these cells must be considered as flattened fibroblasts.

The work done on the differentiation of fibroblasts into serosal cells seems to me inconclusive. That the normal serosal lining cell has a different reaction to vital dyes from that manifested by the fibroblast is undoubted, but no effort has yet been made to determine whether the cells which have formed around foreign bodies or those which have recovered denuded areas of peritoneum give reactions to vital dyes similar to those reported here for serosal cells. It seems, therefore, that the evidence so

far obtained tends to indicate that the serosal cell is a specific cell-type and is not a transformed fibroblast, in the sense of being interchangeable morphologically and physiologically.

The relation which the germinal epithelium bears to the ovary and to the cells lining the general peritoneal cavity has been extensively studied. In the course of these investigations there have developed two principal questions, both of which must be considered in the light of the reactions which these groups of cells manifest towards vital dyes. In the first place, are the germinal epithelial cells closely related to the cells lining the general peritoneum, or are these two groups entirely different genetically and functionally? In the second place, to what extent do the follicular cells, interstitial cells, and definitive ova have their origin in these cells which constitute the ovarian envelope?

Waldeyer ('70), after long and careful study of many species, decided that the cells of the general peritoneal mesothelium were in early stages entirely similar to, and continuous with, the germinal epithelial cells. But later in the developmental history, all of these cells except those over certain specific areas, differing in different species, were destroyed or desquamated and their place taken by subjacent connective-tissue cells. He found that in the amphibia the cells of the general serosa retained their primitive characters in wider distribution than in the mammals, and so he considered that the peritoneal sac might be thought of as fundamentally a reproductive pouch which gradually was narrowed until, in mammals, this property was retained only by the cells of the ovarian envelope.

Gatenby's work ('16) on the frog, in which he found that the general peritoneal lining cells could bud off and produce ova, is in support of Waldeyer's idea. But, on the other hand, many observers have considered the cells covering the genital ridge as being differentiated out of the general coelomic layers.

Neumann ('75) found that there were interesting interrelationships between the cells covering the ovary and those lining the general peritoneal cavity in the frog. He considered them genetically identical, because they both arose from the low

cylindrical layer of cells which lines the peritoneal cavity during its formation from the coelomic layers. Later, the special characteristics of the germinal epithelium are developed in connection with their further differentiation into sexual elements. Furthermore, he found that the flat serosal cells of other areas in the peritoneal cavity of frogs may be transformed into typical germinal epithelium when sexual maturity is reached.

MacLeod ('80) suggests that the peritoneal epithelium also undergoes modifications in other regions, e.g., spleen and pancreas, and Kolossow ('93), in his careful studies of the normal peritoneal epithelium in both mammals and amphibia, comes to the conclusion that the germinal epithelium of the ovary is a part of the true layer of peritoneal lining cells, which, owing to specific stimuli, has undergone a certain amount of differentiation. He also found certain differences in the cells covering the spleen in mammals and in those covering the stomach of amphibia.

Coert ('98) believes that the germinal epithelium is formed from the general coelomic epithelium and denies its specificity in Waldeyer's sense, but agrees with him that the germinal epithelium gives rise to primordial germ cells.

Van Beneden ('80), Allen ('04), Sainmont ('06), working with the cat, Firket ('14), ('20 a), using the chick, and others have come to the conclusion that the germinal epithelium of the ovary is a part of the peritoneal mesothelium which has become cylindrical. Even Waldeyer in his more recent work has modified his earlier opinions indorsing the conclusions of Coert. In general all modern work has tended to establish the genetic continuity of the general peritoneal serosa and the germinal epithelium; whatever the factors may be that determine the specific changes which have been found in the cellular layers, particularly of those over the ovaries, but also to some extent of those covering the spleen, stomach, etc., they must be associated with some stimulus coming from the structures over which the changes take place.

The results of the vital staining of the germinal epithelium and the cells of the general peritoneal lining indicate a certain similarity in the reactions of these two groups of cells. There

are gradations between the intestine, body-wall, diaphragm, omentum, and the spleen; the latter approaching the type of staining manifested by the ovary more closely than the others. These cells have in common the localization of the dye in a particular part of the cytoplasm of the cell, and while there is no direct evidence that similarity in the storage of vital dyes represents similarity in physiological function, yet it is permissible to consider such findings suggestive. The extension of such studies to species such as the frog would be helpful because there is evidence suggestive of a greater similarity between the general serosal lining cells and the germinal epithelium in these species.

With regard to the second question concerning the relationship between the germinal epithelium and the internal structures of the ovary, a very large amount of work has been done. Elaborate reviews of the literature have been given by Firket, Coert, von Winiwarter, and others, and it is entirely unnecessary to repeat these here. In brief, it has been accepted by most workers that the medullary cords are downgrowths of the germinal epithelium, and many also believe the so-called cords of the second proliferation are likewise derived from the germinal epithelium. The question about which most of the discussion has been centered is the relation which the germinal epithelium bears to the definitive ova.

Waldeyer ('70) found large numbers of cells in the germinal epithelium which he interpreted as young germ-cells, and concluded that these cells were developed entirely from the cells of the ovarian envelope. This view has been supported in general by many workers, among whom are von Winiwarter ('01), von Winiwarter and Sainmont ('09), Sainmont ('06), Lane-Claypon ('06), Gatenby ('16), and von Berenberg Gossler ('12). In this way a general school has been developed whose principal belief is the origin of the definitive ova either directly from the germinal epithelium or from the cellular cords which have been developed as downgrowths from this superficial layer of cells.

On the other hand, Rubaschkin ('07, '12) and Swift ('14) have strongly supported the theory, advanced by Nussbaum ('80, '01), that the primordial germ-cells do not originate in the germinal epithelium, but come from cells which have not given

up their embryonic character and have not been differentiated into any especial somatic structures. Eigenmann ('91), Beard ('04), and Hoffmann ('92) have also supported this view. Swift ('15) derives the primordial germ-cells from a particular part of the germ-wall entoderm at the edge of the area pellucida during the primitive-streak stage. When this area becomes vascularized by the extension of the mesoderm, these cells gain entrance to the circulation and settle out in the region of the developing gonad. He agrees that the medullary cords are formed from downgrowths of the germinal epithelium, but considers the cords of the second proliferation as formed by rapidly proliferating, primordial germ-cells or oogonia. Finally, the oogonia form the definitive ova, while the cells derived from the germinal epithelium present in the cortical cords become follicular epithelium.

A third group of workers have assumed that there are two sources for the definitive ova, one the primordial germ-cells which furnish a few ova, while a second generation of germ-cells develop from the germinal epithelium. This view has been supported by Felix ('06), Allen ('04), Dustin ('07), and particularly by Firket. Firket ('14, '20 b) has described the early stages in the chick and rat, and in both species he finds the total number of the primordial germ-cells far too few to permit of their being considered the sole source of definitive ova, so that he is forced to assume an additional development of ova from the germinal epithelium. Using his two species as types of the bird and mammal, he suggests that this process may be a phylogenetic recession.

The discussion of the origin of the definitive ova from the germinal epithelium deals almost entirely with changes which take place during embryonic life, and hence, in mammals at least, would be most difficult to examine by the vital staining technique. However, it is well known that in some species there is formation of ova after birth, and in these it seems that the vital staining of the germinal epithelium might prove a useful adjunct to the study of the question regarding the origin of the definitive ova. My experiments, being confined so far to the

rabbit, do not offer any assistance in settling this point. On the other hand, the similarity between the reactions displayed by the cells of the germinal epithelium and the general serosal lining cells towards vital dyes suggest a closer relationship between these two groups of cells than has usually been assumed.

SUMMARY

1. The serosal lining cells have been found to store vital dyes in a very characteristic manner. The two most striking manifestations of this reaction consisted in the localization of a concentration of dye-granules in a circumscribed area of the cytoplasm of each cell and in the formation of a perinuclear rosette.

2. Mesothelial cells from different areas of the peritoneal surface presented certain peculiarities in their reactions to vital dyes which sufficed to classify them into groups, while they still conformed to the general characteristic type.

3. The variations noted in the cells from different areas of the peritoneal surface consisted in differences in the amount of dye stored, the characteristics of the perinuclear rosette, and the orientation within the cell of the localized collection of dye-particles. The cells covering the intestine usually contained the least amount of dye, while those of the splenic mesothelium and the germinal epithelium of the ovary contained the largest amount.

4. The germinal epithelium was found to store vital dyes in an especially characteristic manner. Each cell contained a round, oval, or cup-shaped mass of granules in the infranuclear zone of the cell; this mass, in well-stained animals, filled the entire portion of the cell between the nucleus and the basement membrane. On the other hand, the perinuclear rosette was found only rarely in the cells of the germinal epithelium.

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PLATE 1

EXPLANATION OF FIGURES

1 Section of the germinal epithelium of a rabbit which had received eight intravenous injections of trypan blue.

2 A binucleate mesothelial cell from the diaphragm of a rabbit which had received twelve intravenous injections of trypan blue.

3 and 4 Mesothelial cells from the spleen of a rabbit which had received ten intravenous injections of trypan blue.

5 A mesothelial cell from the omentum of a rabbit which had received six intravenous injections of trypan blue.

6 A mesothelial cell from the spleen of a rabbit which had received twelve intravenous injections of carmine.

7 A mesothelial cell from the spleen of a rabbit which had received eighteen intravenous injections of carmine.

8 Mesothelial cells from the diaphragm of a rabbit which had received twenty-five intravenous injections of carmine.

Fig 1 (x 2180)

Fig. 2 (x 925)

Fig 3 (x 925)

Fig. 4 (x 925)

Fig. 5 (x 925)

Fig 6 (x 925)

Fig. 7 (x 1300)

Fig. 8 (x 1300)