The sustaining infusions delivered by the pump to maintain these levels were calculated on the basis of the estimated renal functions. Under these conditions, equilibrium was achieved with inulin shortly after 60 minutes and with *p*-amino hippuric acid within 30 minutes.

Glomerular filtration rate and renal plasma flow, as measured by the technics just described, were compared with simultaneous measurements by the usual method involving 3 serial urine collection periods. Four experiments in 3 normal young adult male subjects are summarized in Table I. Excellent agreement between the 2 technics was obtained when the averages of 3 periods are considered. The infusion technic, however, appears to yield more consistent individual values. The variations noted among the serial standard clearances are presumably the result of inaccuracies in urine collection.

Comment. A simple technic for the measurement of glomerular filtration rate and renal plasma flow has been devised. It requires a constant intravenous infusion pump and a minimum of blood samples and chemical analyses. It does not require urine collection and for this reason is simpler and more accurate. The method can be utilized for repeated serial observations during 24-hour periods.

Whether this technic can be adapted to the measurement of the maximal rate of tubular secretion or reabsorption of substances excreted only by the kidneys has not yet been established. It will also be necessary to determine how accurately the method can reflect acute changes in renal function and the effect of acute changes in the volume of body fluid compartments upon the measurements.

Finally, it may be noted that this method could be applied to the measurement of the "clearance" of other substances by other organs, provided the substance under study is excreted or metabolized only by the organ in question.

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Reaction of the Rat Peritoneum to Acid Colloidal Pigments.

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It is now well known that many types of foreign particulate matter are rapidly removed from the peritoneal cavity; this is particularly true of substances in acid colloidal form. Part of such a material is discharged into the lymph stream while the remainder is taken up by macrophages from adjacent connective tissues. MacCallum[1] and others have studied the free cells which appear in peritoneal fluid under these conditions, but most investigators have devoted but little attention to the peritoneum itself. In these investigations, an attempt is made to follow changes in the peritoneum and to note differences in its regional response to various acid-colloidal pigments.

Materials and Methods. Four groups of inbred albino rats, 67 in all, were used in these experiments, each group being composed of individuals of nearly the same age (within one week); all animals weighed 150-190 g at the time of the first injection. One group was intraperitoneally injected with each of the following pigments: trypan blue, Biebrich's scarlet, India ink, and Chlorazol black E (Erie black GXOO). These materials were made up as 2% solutions in distilled water. Animals were given daily injections of 1-3 cc over periods of time ranging from 1-75 days; they were killed 18-24 hours after the final injection. Tissues were fixed in Bouin, Helly or Regaud's solutions, dehydrated in alcohol

[1] MacCallum, W. G., *Bull. Johns Hopkins Hosp.*, 1903, **14**, 105.

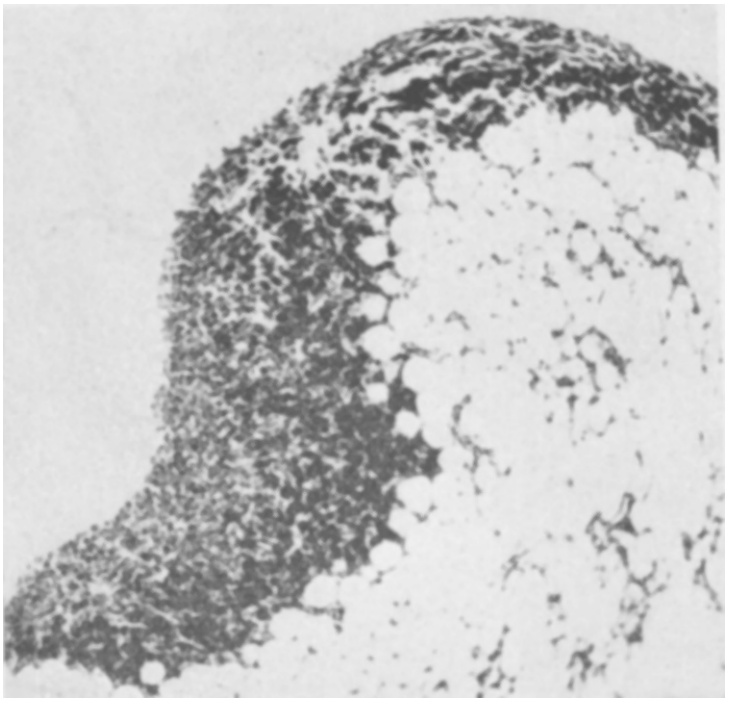

FIG. 1.

Peritoneum of the pelvic fat body. Note the thickening of peritoneum due to collection of free histiocytes and macrophages. The thickest portion (on the left) is completely denuded of mesothelial covering. Photomicrograph × 100.

All unretouched photomicrographs reproduced herein are from tissues of animals injected with Clorazol black E.

and dioxan, embedded in paraffin and sectioned at 6 micra. The sections were stained with hematoxylin-eosin or Mallory's triple combination.

Observations and Discussion. The microscopic features of normal peritoneum are already well known having been fully described by Seifert,[2] Webb,[3] and others. Histologically, it is composed of a continuous surface layer of mesothelium which is closely applied to a subjacent connective tissue lamina. This connective tissue layer may form an external (serous) covering for various abdominal organs or may be continued deeply into extra-peritoneal fat or areolar tissue. As will be indicated later, this extra-peritoneal connective tissue has the same reactive potentialities as the deeper layer of peritoneum proper.

It has been previously noted by Addison and Thorington[4] that the type of free cell response to various pigments is largely dependent on the material used. The present investigation indicates that the same is also true for histological reactions in the peritoneum itself. Of the pigments employed, least reaction followed administrations of Biebrich's scarlet; there were progressively greater responses in the order named, after injections of trypan blue, India ink, and Chlorazol black E.

When pigment solutions are introduced in large doses (2-3 cc per day), ascitic fluid almost invariably collects in the peritoneal cavity. This fluid is thick and syrupy in consistency and fresh smears indicate that it contains an abundance of pigment-filled

[2] Seifert, E., v. Möllendorff's *Handbuch der mikroscopischen Anatomie des Menschen.*, 1927, **6**, 340.

[3] Webb, R. I., *Am. J. Anat.*, 1931, **49**, 283.

[4] Addison, W. H. F., and Thorington, J. M., *Anat. Rec.*, 1918, **14**, 467.

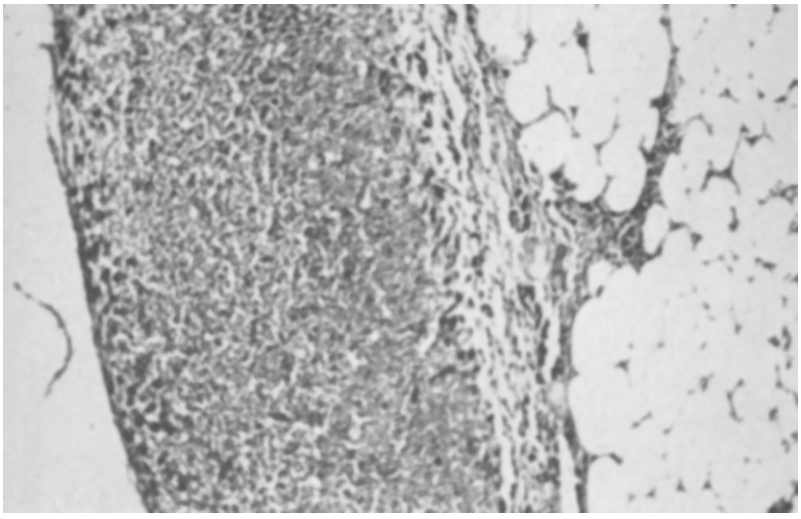

FIG. 2.
Mesentery, showing localized collection of free cells. In this mass there is a central necrotic area surrounded by a zone of intact macrophages. There is partial desquamation of mesothelium. Photomicrograph × 100.

macrophages, lymphocytes of all sizes, granulocytes, erythrocytes, and detached mesothelial cells.

Peritoneum as a whole reacts to injections of particulate matter by becoming colored and thickened; the degree of reaction is dependent on the type of material used and on the region examined. There is a minimum of response in that portion which forms serosa for organs of the gastro-intestinal tract; typically, its tissues are left almost undisturbed. The remaining peritoneum, however, is considerably thickened by collections of histiocytes and pigment-filled phagocytes in the sub-mesothelial connective tissue. Especially after chlorazol injections, the mesothelial cells round up, and, on occasion, separate from their fibrous bed. In such denuded regions peritoneal connective tissue is in direct contact with the peritoneal cavity and its contents (Fig. 1).

In almost all instances, pigment granules can be found within the mesothelial cell cytoplasm. The granules are of small size and rather uniform diameter. This is in sharp contrast to the inclusions of active phagocytes which appear in the sub-mesothelial connective tissue and in ascitic fluid. These macrophages contain pigment inclusions which are highly variable in size and number and which appear to be in all stages of coalescence.

If the experimental animals are subjected to daily injections for 2 or more weeks, some sub-mesothelial phagocytes will become distended by a single, densely colored pigment mass. Such condensation is never encountered in mesothelial cells, either in the attached condition or when they are found free in the peritoneal fluid. Particularly in the latter state, these free cells almost invariably show signs of disintegration. Cunningham[5] has noted that the ovarian germinal epithelium typically contains more granules than other portions of the mesothelial lining and believes that this pigment collection is phagocytic in nature. In these experiments, however, no signs of condensation or coalescence was seen in mesothelium from any region.

The mesentery reacts to injections of particulate matter in the manner described above, but presents several special features which might be mentioned in passing. More than in any other peritoneal region the mesenteric collections of sub-mesothelial phagocytes tend to become localized in nature. Sometimes these collections increase to considerable size

[5] Cunningham, R. S., *Am. J. Anat.*, 1922, **30**, 399.

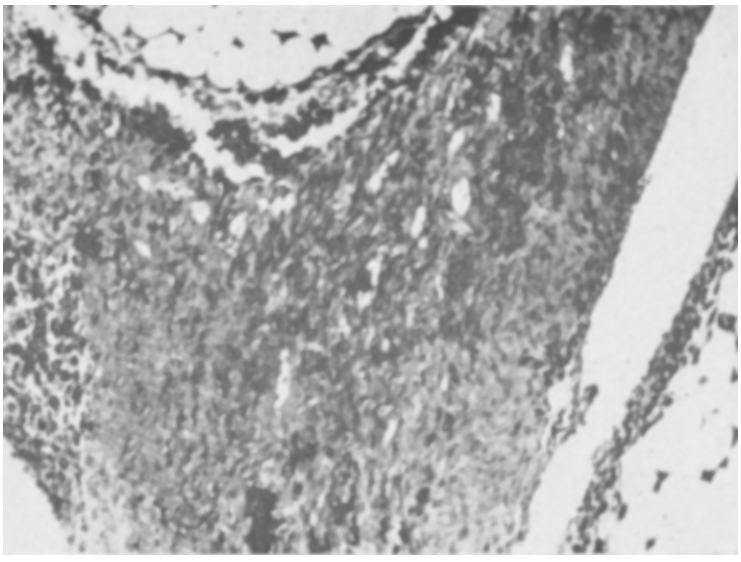

FIG. 3.
Omentum. Fibrous reaction in peritoneum. Note macrophages intermixed with the fiber bundles. Photomicrograph × 100.

and can be located grossly; in which case they are composed of a central necrotic mass of pigment granules and cell debris, surrounded by a zone of intact macrophages embedded in fibrous stroma (Fig. 2). This latter coat may become especially thickened by copious formation of granulation tissue.

The omentum of similarly treated rats has been previously described by Baillif,[6] but further investigations, particularly those employing injections of Chlorazol black E, have produced even more striking response than heretofore reported. Animals which are exposed to this dye for more than 2 weeks' time are prone to develop granulomatous masses; within these masses appear variable numbers of foreign-body giant cells which may or may not contain crystals. It is interesting to note that these crystals are not composed of the injected pigment but of an iridescent yellowish-green material of unknown composition. Occasionally, the chlorazol appears to excite a pronounced fibrous reaction which results in formation of firm, angular masses which are readily visible to the naked eye (Fig. 3).

The diaphragm shows particularly marked response to injections of colloidal pigments. As previously reported by Mackmull and Michels,[7] Simer,[8] and others, this musculofibrous membrane is an important site for removal of particulate matter from the peritoneal cavity. Especially after injections of India ink and chlorazol black E, the diaphragmatic lymph channels are markedly dilated, while the fibrous layer of peritoneum is thickened by collection of histiocytes and macrophages. No indications of stomata such as described by von Recklinghausen[9] and later by Allen[10] were seen in our preparations. Great numbers of mesothelial cells may become freed from the diaphragmatic peritoneum in response to acid-colloidal pigments. In this particular organ, islands of laminated mesothelium appear in the normally simple covering tissue; from these regions individual squamous elements are freed and discharged into the peritoneal cavity (Fig. 4).

The passage of pigment-filled macrophages

[6] Baillif, R. N., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 409.

[7] Mackmull, G., and Michels, N., *Am. J. Anat.*, 1932, **51**, 3.

[8] Simer, P. H., *Anat. Rec.*, 1944, **88**, 175.

[9] Recklinghausen, F. T. von, *Die Lymphgefässe und ihre Bebeihung zum Bindgewebe*, 1862, Berlin, Hirschwald.

[10] Allen, L., *Anat. Rec.*, 1936, **67**, 104.

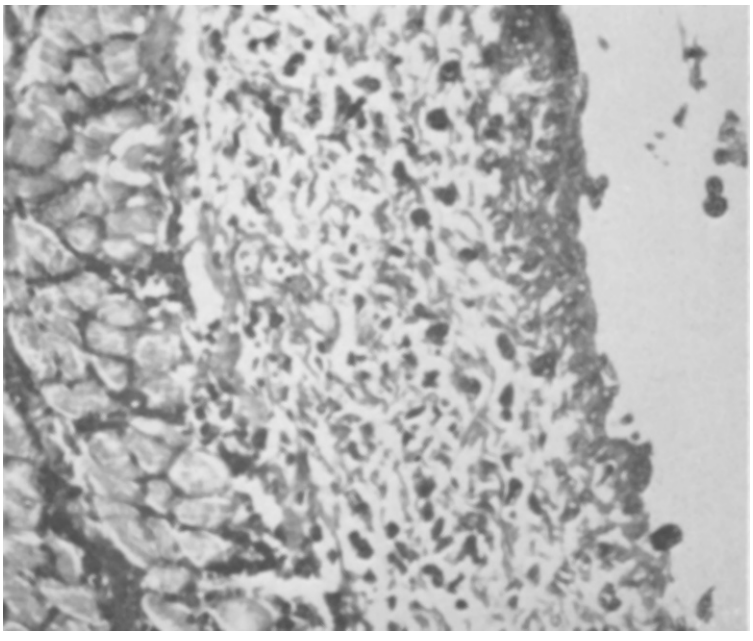

FIG. 4.

Section of the diaphragm. Mesothelium is lacking on a portion of the peritoneal surface (lower right); a macrophage can be seen traversing this denuded area. Other portions of its peritoneal surface appear to be covered by a laminated mesothelial covering (upper right). Macrophages and free mesothelial cells can be seen in the peritoneal cavity. Photomicrograph × 100.

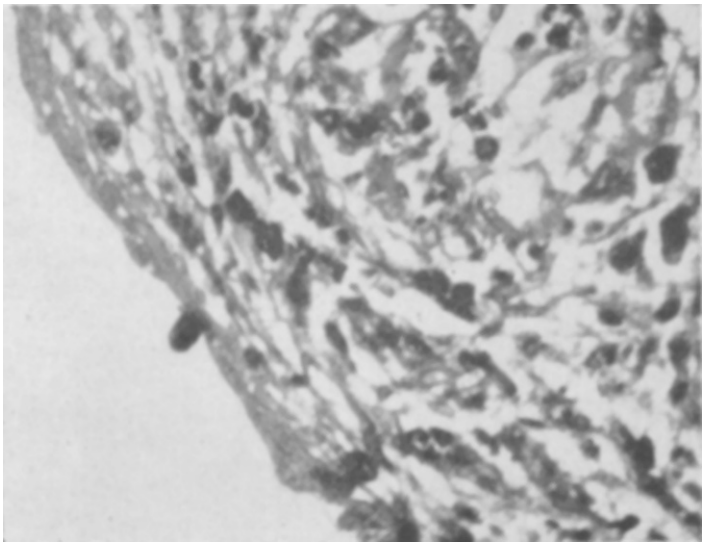

FIG. 5.

Mesentery. Note the collection of macrophages and histiocytes in the fibrous layer. In this case, the mesothelium is intact and a macrophage is passing between adjacent covering cells. Photomicrograph × 250.

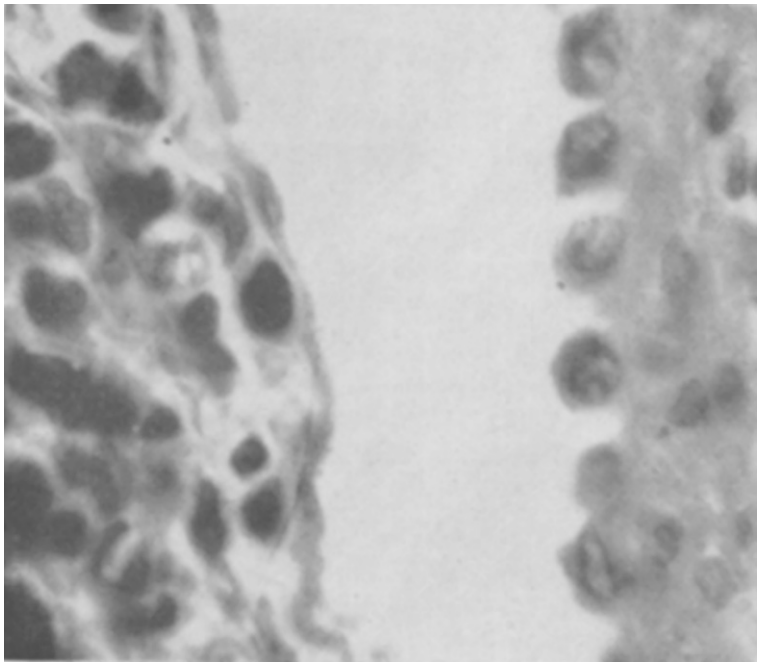

FIG. 6.
Portion of splenic capsule (on the right) and adjacent omentum (on the left of the photograph). Note the rounded mesothelial cells and lack of sub-mesothelial phagocytes in peritoneum of the splenic capsule, in contrast to squamous mesothelium and numerous macrophages in that of the omentum. Photomicrograph × 960.

through peritoneum is frequently seen. The majority escape from denuded areas such as those noted above, but the presence of an intact mesothelium does not offer insuperable barrier to their progress (Fig. 4 and 5).

The peritoneum covering organs of the gastro-intestinal tract is little affected by the presence of acid-colloidal pigments. Its surface layer remains a flattened mesothelium while the subjacent connective tissue lamina is but lightly infiltrated with macrophages. The peritoneum in relation to capsules of the liver and spleen is usually marked by formation of cuboidal mesothelial cells but there is little infiltration of phagocytes in the connective tissue lamina (Fig. 6).

Summary and Conclusions. Rat peritoneum reacts to the presence of various acid-colloidal pigments by thickening, primarily due to the accumulation of sub-mesothelial histiocytes and macrophages. The surface mesothelial cells may round up, and separate from their connective tissue bed. Pigment-filled macrophages escape through these denuded regions and pass into the peritoneal cavity; the same cells are sometimes seen to insinuate themselves between adjacent intact mesothelial cells as well. Localized collections of free cells may appear in the peritoneum of the mesentery and omentum; when large, these masses typically show necrotic centers.