apparently gave some immunity in the vaccinated group at institution Y, but none at X.

V-2 produced a slightly, but perhaps not significantly, greater antibody-response than did V-1, as judged by complement-fixation and neutralization-tests. It is quite probable that subclinical infections accounted for some of the increase in antibodies. It also appeared that the response in complement-fixing antibodies produced by the two vaccines was considerably less in degree than the response following infection.

Summary. Studies were conducted at two large institutions during an outbreak of influenza A which was proven by complement-fixation tests. Persons at both institutions received a living virus and complex formalinized vaccine previous to the outbreak. An epidemiological survey revealed some protection at one institution, but none at the other. There was no obvious difference in the prophylactic efficacy of the two vaccines in the relatively small group of 829 individuals studied.

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Reaction of the Rat Omentum to Injections of Particulate Matter.

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It has been known for many years that certain cells widely distributed throughout the body have a special property which enables them to collect and store foreign particulate matter. On the basis of this common property it is now rather generally believed that these cells should be considered as belonging to a single system, the reticulo-endothelium. The critical reviews of Maximow[1] and Jaffe[2] are the most useful of the numerous articles available concerning the distribution, morphology and reactive characteristics of this system.

In spite of the many studies carried out in this field, the special

[1] Maximow, A. A., The macrophages or histiocytes, Section XIX in Cowdry's *Special Cytology*, 1932, p.711.

[2] Jaffé, R. H., The reticulo-endothelial system, Section XV in Downey's *Handbook of Hematology*, 1938, p. 974.

physiologic properties which enable certain cells to collect and store foreign particles are still incompletely understood. In the experiments herewith reported special emphasis was placed on the cellular changes which take place during this process. An effort was also made to differentiate between phagocytosis proper and simple transmission of particles by cells, and special attention was directed to changes which occur in the Golgi apparatus of cells engaged in phagocytosis.

Materials and Methods. Seventy-nine albino rats were injected intraperitoneally with trypan blue, lithium carmine, or India ink suspended in saline solution or distilled water, in dosages varying from 0.2 to 137 cc per animal. After completion of the experimental period the animals were sacrificed under ether anesthesia. Early phases of phagocytosis were most numerous and most readily studied in animals killed 18-24 hours after a single injection of 0.5 to 1.0 cc. Later phases were best studied in animals given daily injections varying from 0.2 to 2.1 cc over periods ranging from 5 to 75 days and killed 18-24 hours after the last injection.

The tissues to be examined were fixed in Bouin's or Regaud's solution, or in Ludford's modification of the Mann-Kopsch solution. They were then dehydrated in dioxan, embedded in bayberry-paraffin, and sectioned at 5 μ. Slides were stained in Regaud's hematoxylin, Heidenhain's azan, or hematoxylin-eosin.

Observations and Discussion. Within 18 hours after the first injection of the particulate material a marked histiocytic response appears in the omental substance. The most striking feature of the reaction is the mobilization of enormous numbers of histiocytes, which are rapidly transformed into macrophages and ingest the incoming pigment.

Particulate matter enters the omentum (1) by surface transmission and (2) via blood vessels.

(1) It has been shown that the majority of pigment granules enter the omentum by passing between the mesothelial cells, only a few passing directly through them. The transformation of the covering cells into macrophages has never been observed. As soon as any particles pass into the omentum the resting histiocytes mobilize and begin to migrate toward the surface, where they are soon transformed into active phagocytes which engulf and store the entering particles (Fig. 1).

(2) Within a few hours after the first injection granules can also be observed in the walls of the smaller omental blood vessels. They pass through the vascular walls and appear in the perivascular

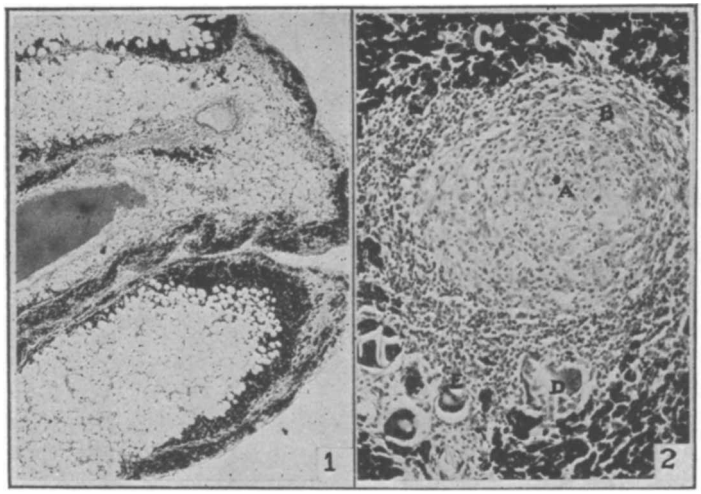

FIG. 1.
Omentum of an animal injected with India ink. Note folding of surface and sub-mesothelial macrophages. × 25.

FIG. 2.
Tasche laiteuse showing: A. Central reticular zone; B. Lymphocytoid zone; and C. Phagocytic zone; D. and E. are foreign body giant cells.

loose connective tissue where they seem to excite the same histiocytic response as do the cells which enter by surface transmission, as evidenced by a cellular migration, transformation of the migratory cells into macrophages, and phagocytosis of the granules. The process leads eventually to formation of dense masses of loaded perivascular macrophages.

Definite reactive changes appear in the tasches laiteuses, particularly after a protracted series of pigment injections. The process culminates in the production of numerous macrophages, which emerge from the periphery of the milky spots. Under these conditions the activated spots consist of three concentric zones which merge into each other: (1) a light central zone made up of reticular cells; (2) a zone packed with cells of lymphocytoid type; (3) an outer zone in which these cells undergo gradual transformation into phagocytes which engulf the free pigment. There is a close correlation between the loss of lymphocytoid characteristics in the nucleus and the assumption of phagocytic power by these cells (Fig. 2).

Cytologic examinations of omenta removed while phagocytosis was taking place show a series of changes in the histiocyte which culminate in assumption of phagocytic power. Under favorable conditions all the changes can be observed in a single section, but

their interpretation and chronological arrangement are naturally subject to error on the part of the observer. This transformation seems to occur as follows:

(1) At the advent of mobilization the ordinary histiocyte increases in size and begins to show cytoplasmic vacuolation. Still more striking is the alteration in the configuration of the Golgi apparatus, first observed by Nassonov.[3] The reticular material of

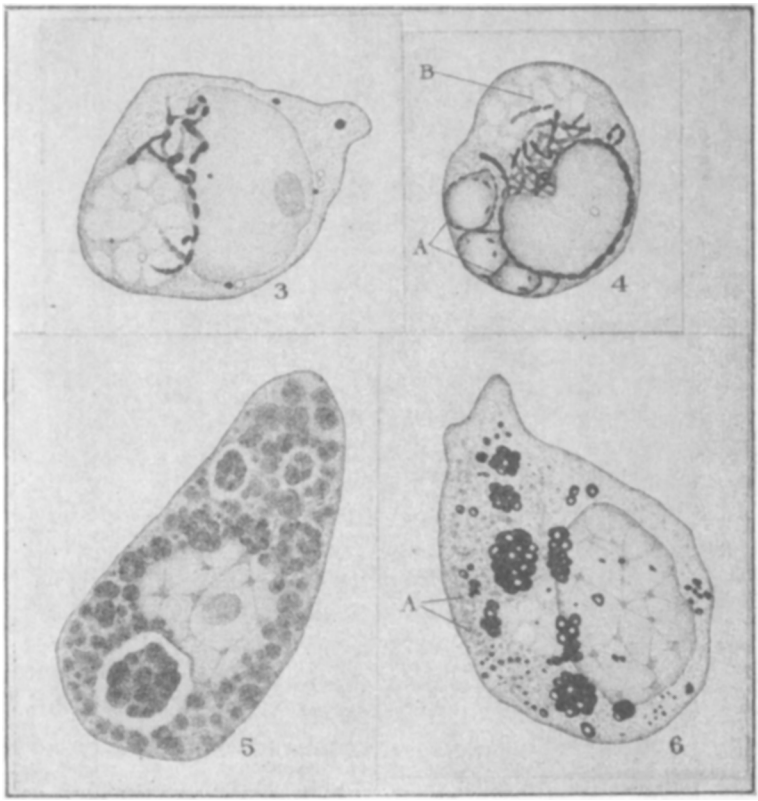

FIG. 3.

Active macrophage showing a complex vacuole with pigment, and fragmented reticular material at its periphery. Ludford's fixation after trypan blue.

FIG. 4.

Macrophage with increased quantity of Golgi material, some of which is distributed about pigment vacuoles (A). B represents newly formed pigment clumps.

FIG. 5.

Macrophage showing numerous India ink granules. Pale rings about the larger masses represent Golgi material in negative. Bouin; Hematoxylin-eosin.

FIG. 6.

Macrophage showing incoming India ink particles (A), and condensing granules which will form a pigment mass. Note that some of the reticular material is enclosed in the mass, showing as pale centers. Bouin; hematoxylin-eosin.

[3] Nassonov, D., *Z. f. Zellforsch u. mikr. Anat.*, 1925, **3**, 472.

the resting histiocyte, which is in the form of a small, closely meshed net applied to the nuclear membrane, increases in size, becomes less closely meshed, and undergoes partial fragmentation. Isolated osmiophilic strands can soon be found distributed throughout the cytoplasm. By the time these changes have occurred, the cell is able to phagocytose particles, as is shown by the occasional presence of pigment granules in the cytoplasm (Fig. 3).

(2) Observations show that when the granule enters the cytoplasm it is soon enclosed in a fluid vacuole, which seems to attract the nearest Golgi fragments. Later the strands become so closely applied to the periphery of the vacuole as to form a continuous osmiophilic shell (Fig. 4).

(3) When two or more of the structures described, each of which is composed of a pigment granule in a fluid vacuole surrounded by an osmiophilic shell, come into contact with one another, they rapidly fuse. This process does not seem to take place prior to the formation of the osmiophilic shell (Figs. 5 and 6). When the contact is established, the pigment granules fuse and the shell disintegrates into discrete droplets deposited in the neighborhood of the developing pigment mass. The Golgi material seems to serve merely as an aid in the process of fusion; there is no indication that any part of it is incorporated in the fused mass.

A study of the many types of cells occasionally found to contain particles of the injected material showed that only during the process of active collection and storage of foreign pigment (true phagocytosis) was there any Golgi reaction to the presence of the ingested particles. The configuration of the reticular matter remained unchanged in the cells which merely transmitted the pigment. The reaction of the Golgi material can therefore be used as an index of phagocytosis.

Summary and Conclusions. The rat omentum, following the injection of particulate matter of various types, undergoes hypertrophy due to accumulation of histiocytes which are transformed into macrophages. The macrophages collect just beneath the surface and phagocytose the material which enters through and between the mesothelial cells. Secondary collections of macrophages appear about the smaller blood vessels and take up the particles which have traversed the vascular walls. The taches laiteuses increase in size and produce numerous macrophages.

With the advent of phagocytic activity the Golgi net increases in size and undergoes fragmentation. The fragments become asso-

ciated with the ingested granules and apparently aid in bringing about their coalescence into masses which are stored by the cells. The changes found in the Golgi material never occur in cells which do not collect and store particulate matter.

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Excretion of Thiamin and its Degradation Products in Man.*

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The development of a simple procedure[1] to determine both thiamin and the pyrimidines in human urine permits the investigation of the possible relationship between the two.

Schultz, Atkins and Frey showed that the rate of glucose fermentation by a yeast is directly proportional to the concentration of thiamin present. In addition to thiamin, the pyrimidines give mol for mol stimulation effect on the rate of fermentation. Such pyrimidines are found normally in human urines. Since the pyrimidine nucleus constitutes an integral part of the thiamin molecule, it is of interest to determine the relationship between thiamin and urinary pyrimidine.

The total fermentation is a measure of thiamin and pyrimidine in the urine. The fermentation after oxidation of the free thiamin is a measure of the pyrimidines; the difference represents the free thiamin. This technic combines the gas method with the initial steps of the thiochrome procedure.

The urinary excretion of the thiamin and pyrimidine of a group of patients was studied under various conditions. Three patients were given a diet completely deficient in B_1 for 10 days. Two of these were normal and one had ileo-jejunitis. Fig. 1 and 2 and Table I illustrate that complete deprivation of dietary thiamin for a period of 10 days changed the thiamin-pyrimidine ratio from approximately 9:1 to 1:9. During this 10-day deprivation period,

* This work was supported in part by the Williams-Waterman Fund.

We are indebted to Dr. Charles Frey, of the Fleischman Laboratories, for his coöperation.

1 Schultz, A. S., Atkin, L., and Frey, C. N., *Science*, 1938, **88**, 547; *J. Biol. Chem.*, 1940, **136**, 713.