de concentration des mesures de *Gini*. Cette étude montre que les résultats sont identiques, parfois superposables pour les données suivantes dans les deux racines: moyenne arithmétique, étendu des classes, médiane.

De ce fait, on peut conclure que les fibres des 2 racines présentent la même épaisseur.

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# LOCATION OF LIPASE ACTIVITY IN THE CHICK EMBRYO

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In the early stages the chick embryo is nourished mainly on lipids and proteins contained in the yolk. It is reasonable to suppose that the enzymes responsible for breaking down these substances play an important part at this stage of development. *Ammon* and *Schütte* [1935], by means of quantitative chemical methods, showed that both the lipase and esterase content of the yolk sac is greater than those of either the embryo or the yolk. In the embryo the content increases steadily and rapidly during development. Similar results have been reported by *Lindwall* [1948] in the sea urchin.

The fact that there is now a histochemical method which renders lipase visible has led us to study its distribution in chick embryos during development.

### *Technique.*

The authors have used the technique of *Gomori* [1945], which shows clearly the sites of lipase activity. Throughout the research the substrate used was a water-soluble ester of stearic acid (Tween 60–G 9096 CJ), a product of the Atlas Powder Co., Wilmington, Delaware. Since the authors have been unable to distinguish between the real lipase (of the pancreas and the stomach) and the esterases of other organs (*Gomori* 1948 and 1949), lipase is used in a general sense, and includes the true lipase and the esterase of the liver, gut, kidney, etc. White Leghorn embryos of various ages were used. The earlier ones were fixed in toto, embedded and cut into serial sections. The older ones were dissected under a magnifying glass and fragments of the various organs were embedded together in the same block, in order to secure comparable results. In each case a piece of rat pancreas was included as a control of the reaction. A control was also used for possible false positive reactions, by incubating sections in a mixture without "Tween".

### *Results.*

In the early stages of development, lipase activity is limited to the endoderm of the yolk sac. A positive reaction is not shown by the primitive streak, Hensen's node, the cephalic process, or any other part of the blastoderm (fig. 1). In the endoderm the most intense and widespread reaction occurs in the large, clear cells with eccentric nuclei. The lipase reaction appears most strongly in a polar position distal to the ectoderm and very close to the yolk. In the yolk adherent to the yolk sac a positive reaction may be seen, either in the interior of giant yolk cells, or between them, extracellularly. Sometimes reactive material appears as bundles of very fine needles (fig. 2). The lipase content of the yolk sac persists troughout development, but the reaction diminishes visibly later. The wall of the yolk sac, in the region of the connecting stalk, is the first place where the reaction decreases, and ultimately disappears.

In the 35-somite embryo, after 3 days' incubation, the reaction is evident in the hepatic rudiment and in the adjacent foregut. In the gut the reaction appears towards the apical pole of the epithelial cells (fig. 3). It is very intense and occurs as a dense granular zone forming a continuous band in the superficial part of the cells. The midgut shows an intense reaction at the level of the intestinal portal. It is interesting to observe that the yolk sac immediately adjacent to the midgut is devoid of reaction (fig. 4), which appears only at a certain distance from the umbilical orifice. The gut reaction persists and increases throughout its hole length (fig. 5). In the rectum of a three-day old chick the reaction is strongly positive. At the end of

Fig. 1

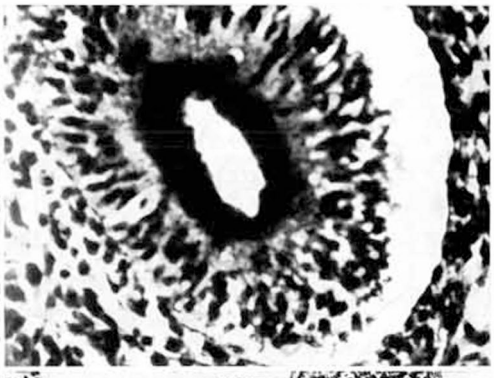
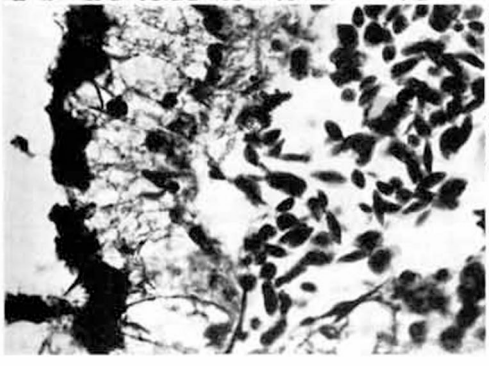


Fig. 3

Fig. 2



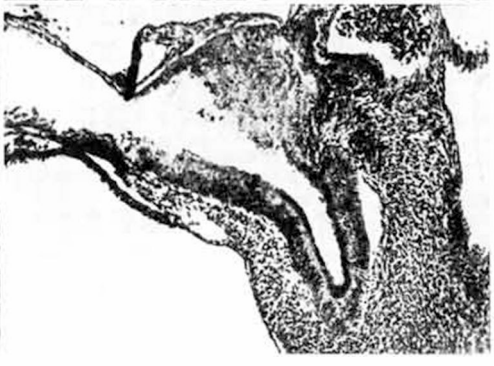


Fig. 4

*Fig. 1.* Chick embryo of three days' incubation. Yolk sac. The histochemical lipase reaction appears as a wide black line in the apical pole of the cells of the vitelline epithelium. × 100. – *Fig. 2.* Chick embryo of 56 hours' incubation. Strong reaction in the vitelline epithelium. Needle-like formations. The vacuoles visible in figs 1 and 2 are seen to be filled with lipid droplets when stained with Sudan III. × 350. – *Fig. 3.* Foregut of chick embryo on the 4th day of incubation. Strong reaction in the apical pole of the lining cells. × 350. – *Fig. 4.* Chick embryo of 4 days' incubation. Communication between midgut and yolk sac. The reaction is clearly visible in the gut lining, disappearing just within the vitelline sac. It reappears in the wall of the sac at a certain distance from the opening. × 100.[1])

the incubation period certain large cells appear in the intestinal villi. These cells are free, spherical and replete with granules showing a positive lipase reaction (figs. 6 and 7). The bursa fabricii, on the other hand, shows a negative reaction throughout its development. The lipase is located in the glandular cells of the forestomach as well as in the cells lining the crypts. The hepatic bud, consisting at this stage of trabeculae with two rows of cells, presents a slight, yet evident reaction in the form of granules situated towards the centre of the

[1]) All sections made by *Gomori*'s technique and counterstained with hematoxylin and eosin.

trabeculae. The common bile duct, cystic duct and hepatic duct (fig. 8) show a marked positive reaction towards the apical pole of their lining cells.

On the third day, the pancreatic bud does not present the lipase reaction, which appears only after the 4th day of incubation. As the embryo develops, the reaction becomes gradually stronger, until by the 18th day of incubation it is the same as in the hatched chick (fig. 9).

In various parts of the body the cytoplasm of the mesenchymal cells exhibits a lipase reaction. Thus, by the 3rd day of incubation, the cephalic mesenchyme contains cells with a positive reaction. This becomes more evident on the succeeding days, especially in the sheath of cells surrounding the notochord. The mesenchymal cells which make up the endocardial cushions show a marked lipase reaction. The reaction is also clearly positive in the precartilaginous blastema of body and limbs. A positive lipase reaction becomes visible in adipose tissue as soon as it differentiates (fig. 10).

The somites show no reaction whatever, apart from that to be seen in scattered mesenchymal cells. The cells of both the cardiac and skeletal muscles are negative, and so is the splenic primordium.

The neural tube gives a slight reaction limited to the white matter of the basal region. This reaction is visible during the first days of incubation but disappears later. In the mesonephros the reaction is of a moderate intensity in the tubules and negative in the glomeruli. In the metanephros the reaction varies being negative in some cases, while in others it is positive in the convoluted tubules after 18 days of incubation.

In the genital ridges the reaction is weak and inconstant. It is negative in the rudiment of the ovary or testis.

There is no reaction in either the amnion or the allantois.

## *Discussion.*

Abundant lipase in the yolk sac, previously demonstrated by chemical methods by *Ammon* and *Schütte* [1935] and now made evident by means of histochemical technics, and its situation at the surface in contact with the yolk, suggests that the cells lining the yolk sac play a part in digesting and resorbing fats, the most important nourishment of the embryo in its early stages. As yet information necessary to judge the mechanism by which these lipids are absorbed

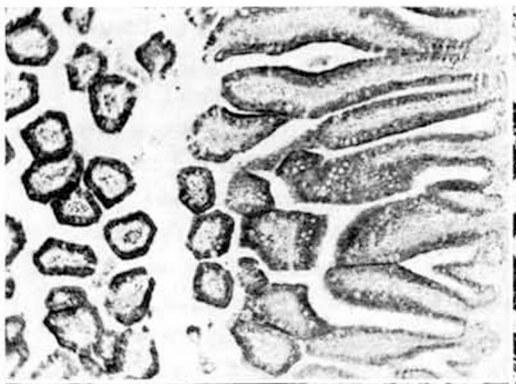


Fig. 5


Fig. 8

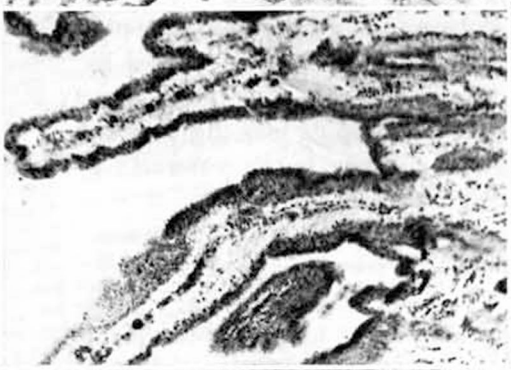


Fig. 6

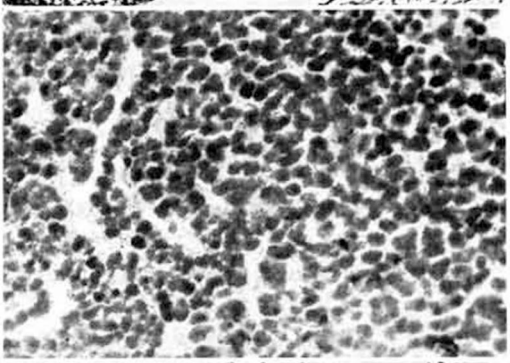


Fig. 9

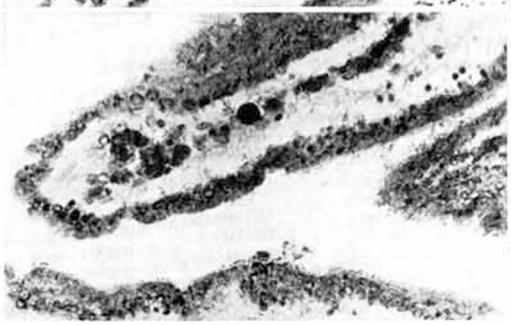


Fig. 7

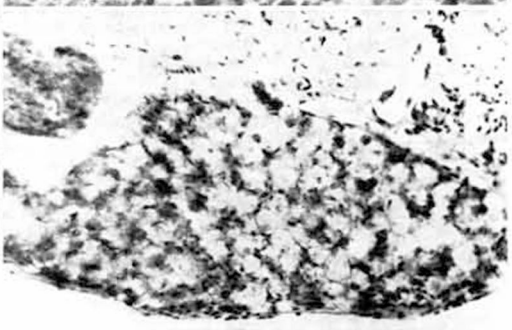


Fig. 10

*Fig. 5.* Small intestine of chick embryo of 18 days' incubation. Lipase situated in the apical pole of lining cells. × 100. – *Fig. 6.* Chick embryo of 18 days. Villus of small intestine. Lipase in the epithelium. Large, free, spherical cells in the axis of the villus, showing a strong lipase reaction. × 120. – *Fig. 7.* The same section as figure 6, under higher power. × 250. – *Fig. 8.* Chick embryo of 4 ½ days' incubation. Strongly marked reaction in the gut (1), common bile duct (2), and cystic duct (3). Less intense reaction in the hepatic duct (4) and in the columns of hepatic cells (5). × 100. – *Fig. 9.* Chick two days after hatching. Pancreas. Lipase reaction in the alveolar cells. × 120. – *Fig. 10.* Chick immediately after hatching. Fat lobule. Lipase reaction in the adipose cells. × 100.

is lacking, but it would appear reasonable to suppose that the lipase of the yolk sac is instrumental in this process.

The gut follows the yolk sac as regards the quantity of lipase content. In the early stages there is a marked difference between the foregut relative to the hepatic and pancreatic buds and the rest of the alimentary canal. The former shows a strong lipase reaction: the later none at all. This suggests a histochemical and functional differentiation.

On the 3rd or 4th day of incubation the hepatic cells show a granular type of lipase reaction coincident with the presence of lipids in the cytoplasm. Similarly in the mesenchyme there is an abundance of lipase at those points where adipose tissue differentiates. The presence of lipase in foetal fibroblasts has been described by *Gomori* [1946].

With suitable tecnique the presence of lipids may be demonstrated in many embryonic cells as, for instance, in the nerve cells at different stages of development. Nevertheless they do not show a positive lipase reaction, which would seem to indicate that the facts contained in them do not undergo the same metabolic process as those in the vitelline endoderm.

In the mesonephros and metanephros the lipase appears to be distributed in accordance with the observations of *Gomori* [1946], who succeded in demonstrating the presence of these enzymes in the kidneys of certain species.

In the present observations the lipase is always seen in the cytoplasm, and never in the nucleus as observed by *Nachlas* [1949], who used a different technique.

These studies upon the distribution of lipase in the embryo point to an early and marked differentiation of the enzymatic activity. *Moog* [1944], studying the distribution of acid and alkaline phosphatase in the chick embryo found that in the initial stage these substances are distributed diffusely, later becoming localised in certain organs and tissues while disappearing in others. She believes that there exists a primary indifferent phosphatase distributed throughout all special organs. The present observations cannot be reconciled with this hypothesis since the distribution of lipase is remarkably precise and limited to certain cells from the very first.

Another fact worthy of attention is the difference in the behaviour of the fundus and connecting stalk of the yolk sac. This is perhaps due to histological and functional differences between these two

regions. *Coppini* [1937] found that trypan blue injected into the yolk stains the fundus, but not the connecting stalk of the yolk sac.

*Summary.*

1. In the blastoderm of the chick embryos up to the third day of the incubation, lipase is to be found only in the vitelline endoderm, the large cells of which show a strong lipase reaction.

2. From the 3rd day a positive reaction appears in the fore- and midgut, and in canalicular and hepatic cells of the hepatic rudiment. Later it also appears in the pancreatic bud, in the rest of the gut and in the glandular stomach.

3. Some mesenchymal cells show a positive reaction which is evident in the perichordal sheeth, in the endocardial cushions, in the precartilaginous blastoma, and in those places where adipose tissue formation takes place.

4. The metanephros and mesonephros show a weak and inconstant reaction.

*Résumé.*

1. Jusqu'au 3e jour d'incubation le blastoderme de l'embryon de poulet ne renferme de la lipase qu'au niveau de l'endoderme vitellin dont les grandes cellules donnent une réaction fortement positive.

2. Dès le 3e jour une réaction fortement positive apparaît dans le tractus intestinal supérieur et moyen, ainsi que dans les épithélia des canaux biliaires et du bourgeon hépatique. Plus tard, le ferment peut être mis en évidence dans le bourgeon pancréatique, dans le reste de l'intestin et la portion glandulaire de l'estomac.

3. Quelques cellules du mésenchyme donnent une réaction positive: gaîne de la notocorde, coussins endocardiques, blastème précartilagineux et les points où se formera du tissu adipeux.

4. Le métanéphros et le mésonéphros ne montrent qu'une réaction faible ou inconstante.

*Zusammenfassung.*

1. Im Blastoderm des Hühnchen-Embryos findet sich bis zum dritten Tage der Bebrütung Lipase nur im Dotter-Entoderm, dessen große Zellen eine starke Lipase-Reaktion aufweisen.

2. Vom dritten Tage ab erscheint eine positive Reaktion im Vorder- und Mitteldarm und in den Zellen der Leber und in den Gallenkanälchen der Leberanlage. Später erscheint Lipase auch in der Pankreas-Anlage, im übrigen Darm und im Drüsen-Abschnitt des Magens.

3. Einige Mesenchymzellen weisen eine positive Reaktion auf, z. B. in der perichordalen Scheide, in den Klappenanlagen des Endocards, im Vorknorpel und an den Stellen, an welchen Fettentwicklung stattfindet.

4. Die Nach- und Urniere weisen eine schwache und inkonstante Reaktion auf.

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