ON THE ABSORPTION OF IRON IN THE ANIMAL BODY.

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THE chemico-physiological relations of iron have been the subject of much speculation during the last fifty years. The chief difficulty in investigating the subject experimentally is the fact that, when iron enters into the composition of organic structures, it becomes 'masked' and can no longer be detected by its ordinary chemical reactions.

The question is one of great practical interest, and Bunge's researches have in recent years done much to unsettle the medical dogma that the iron contained in drugs enters directly into combination with the red corpuscles to form hæmoglobin.

In 1890 an investigation on the formation of blood corpuscles in larval amphibia led me to conclude that hæmoglobin is formed from nuclein (the chromatin of histologists), and the iron-holding character of other nucleins has been demonstrated by Bunge[2], and Zaleski[3]. The discovery of micro-chemical methods[4] for detecting iron in cells has aided me in establishing the generalisation that the most important of all elements in the life of every cell is an iron-holding compound. The prozymogens, if not the zymogens themselves, also contain iron.

I regard Bunge's theory of the direct conversion of iron-containing nucleins in the food (hæmatogen) into hæmoglobin as extremely doubtful, but the whole question of the synthesis of organic iron compounds is in an uncertain state, chiefly owing to our ignorance of the constitution of the nuclein molecule. There are, however, certain allied questions which can be more readily answered, and I have

[1] The expenses of the investigation of the absorption of organic compounds of iron (chromatins) were generously defrayed by a grant from the Elizabeth Thompson Science Fund.

[2] *Zeit. f. physiol. Chem.* Vol. IX. p. 49, 1885.

[3] *Ibid.* Vol. X. p. 453, 1886.

[4] Macallum. *Proc. Roy. Soc.* Vol. L. 1891.

attempted in the following pages to determine first whether or not inorganic compounds of iron are absorbed, and secondly whether certain organic compounds of iron are absorbed; this second part of my paper must however be regarded as a preliminary communication, as my work in this direction is still incomplete.

I. On the Absorption of Inorganic Iron Compounds.

The literature of this subject is very abundant; it consists largely of clinical records; the only papers however which I will mention are those which embrace observations on the quantitative estimation of iron ingested and excreted. In these the difference between the amounts is relied on to show whether iron salts are absorbed or not. The principal researches of this nature are those of Kletzinsky[1], Hamburger[2], Gottlieb[3], Kunkel[4], Kumberg[5], Busch[6], Marfori[7], Coppola[8] and Stender[9].

A careful perusal of these papers will convince the enquirer that the methods adopted are not calculated to answer the question, and that the various observers obtained different results.

In none of the series of experiments referred to, however, was the micro-chemical method employed to any extent except in those of Stender, although Kunkel's experiments on the livers of mice fed with iron indicate how valuable such a method would be in results, and it seemed to me that it would be easy to determine with it whether there is absorption of iron salts, and if so, in what manner and through what physiological agents it takes place.

Influenced by these views as to the value of the micro-chemical method, I made a number of experiments with guinea-pigs, kittens, lake-lizards and *Amblystomata*, using different preparations of iron and administering them either with or without food in various doses. At different intervals, after administering a preparation, or during the course of feeding with the preparation, the animal was killed, the abdomen

[1] *Zeitsch. d. Gesellsch. der Aerzte zu Wien*, 1854, ii. 281.

[2] *Zeitsch. f. physiol. Chem.* Vol. II. 1878, p. 191.

[3] *Arch. f. exp. Path. u. Pharm.* Vol. XXVI. p. 139, 1889; *Zeitsch. f. physiol. Chem.* Vol. XV. p. 371, 1891.

[4] *Arch. f. d. ges. Physiol.* Vol. L. p. 1, 1891.

[5] and [6] Kobert. *Arbeiten des Pharm. Inst. zu Dorpat.* Vol. VII. 1891.

[7] *Arch. f. exp. Pathol. u. Pharm.* Vol. XXIX. p. 212, 1891.

[8] *Rendiconti della R. Accad. dei Lincei.* Vol. VI. p. 362, 1890.

[9] Kobert. *Loc. cit.*

opened, parts of the intestine, liver, spleen and kidney were removed, put directly into 95 per cent. alcohol or, after hardening for ten minutes in a saturated solution of corrosive sublimate, into alcohols successively of 50, 70, and 95 per cent. strengths. For control purposes teased-out portions of the fresh mucous membrane of the intestine were treated with ammonium sulphide and examined under the microscope. After the hardening was completed in alcohol, sections were made of the various parts either by the free hand (liver and kidney), or by the paraffin or celloidin methods. Sections made by the paraffin method from tissues hardened in corrosive sublimate were fixed on the cover slip by Gaule's method, and the paraffin having been removed by the usual process they were immersed in a mixture of equal parts of solutions of hydrochloric acid and potassic ferrocyanide of 0·5 and 1·5 per cent. strengths respectively. Here they were allowed to lie for about ten minutes, then they were washed in distilled water, dehydrated, cleared in cedar oil and mounted in benzole balsam. Sections made with the free hand, or with the celloidin method, were either mounted on the slide in a mixture of glycerine and ammonium sulphide and examined under the microscope, or placed in the ferrocyanide mixture for ten minutes, then carefully washed in distilled water, dehydrated in alcohol and, after clearing in oil of cedar, mounted in benzole balsam. Portions of the intestine hardened in alcohol were also put in a mixture of alcohol and ammonium sulphide in the relation of 5 to 1. Material hardened in corrosive sublimate, on account of the presence of this reagent in the tissues, gave no useful preparations with ammonium sulphide, but they furnished, nevertheless, instructive ones when treated with the ferrocyanide mixture. In studying the preparations made with ammonium sulphide, I teased out portions of the mucosa and mounted them in a mixture of glycerine and ammonium sulphide. The glycerine prevents largely the evaporation of the sulphide, and such preparations may be kept weeks and even months. In the case of the liver, spleen and kidney teased-out preparations yielded nothing of value, but sections of alcohol material, made sufficiently thin with a little practice by the free hand, were useful when mounted in the glycerine sulphide mixture. I have found that material hardened in alcohol furnished the best preparations, and that it gave results not equalled by those given by material fixed with corrosive sublimate, or those obtained by the use of ammonium sulphide on fresh material. The latter method is apt to lead to error, since the living or non-hardened tissues are slowly penetrated by ammonium sulphide, and when the penetration

does take place, the cellular elements are more or less altered, giving confusing results. Alcohol has the advantage that it hardens rapidly and does not extract the salts of iron which are in the tissues in an albuminate form. In order to prevent diffusion of iron salts from without into the mucosa of the intestine, the latter, laid open immediately after removal, was in every case quickly washed free from adherent food matters and then dropped into alcohol.

In guinea-pigs there is absorption of iron in the intestinal mucosa. This is readily seen in well-fed animals, less readily so in those whose stomachs and intestines are almost empty of food. The intestinal mucosa, after treatment with alcohol and when tested with ammonium sulphide, acquires a more or less dark colour, due to the formation of sulphide of iron which, under the microscope, is seen to be limited to the sub-epithelial portions of the tips of the villi. On closer examination the iron is found to be deposited in leucocytes which, in their disposition, form together a cap as it were for the extreme end of the lacteal vessel. The dark green reaction is not uniformly diffused through each cell, the nucleus being free from it, while in addition to that present in the cytoplasm as a whole there are masses in it which yield a greater intensity of colour. The leucocytes are not as numerous immediately under the membrane on which the epithelium rests. They may occasionally be found between the epithelial cells of the tips of the villi, not, however, as much loaded with iron as those are which are found about the end of the lacteal. The iron of these cells originates from the food in great part, for, if the animal be kept without food for a week, the tips of the villi give but a feeble reaction. What is present in such preparations is derived from the bile, and this was shown by the results obtained from feeding guinea-pigs with egg-yolk. The latter according to Bunge contains but a trace of inorganic iron—and when it is fed to the animal but a feeble reaction for inorganic iron is obtainable in the villi. When, however, a trace of ether is added to the yolk given, the amount of bile poured into the intestine becomes greater, the absorption more vigorous, and then one finds the tips of the villi give a marked iron reaction almost as distinct as that present in the animal fed on its ordinary diet. This iron must therefore be derived from the bile. In all well-fed animals the iron reaction obtained is the more marked the nearer the part examined is to the pyloric opening, and at a distance of from seven to ten inches from it it is usually absent altogether.

In ordinary guinea-pigs it is indeed very seldom that one finds inorganic or albuminate iron in the epithelial cells themselves. In

order to meet the objection that possibly the iron in the lymph cells is that in the process of excretion, and also to determine how the iron compounds reach these elements, I fed several animals, which had fasted for about four days, with various preparations of iron, but using, for this purpose specially, some of the commercial "peptonate" of iron of which about 100—200 mgrm. were administered *per os* daily to each animal. The intestinal mucosa of animals so fed for three days became black in the ammonium sulphide solution, and on examination with the microscope the reaction, as before, was almost confined to the tips of the villi, which were all similarly affected down to the distal end of the small intestine. It was further found that the epithelial cells themselves covering the tips were loaded with iron, the leucocytes were massed below in great numbers and a large number had wandered between the epithelial cells in such a way as, in many of the villi, to displace and distort the cells. In very thin sections treated with the ferrocyanide mixture and mounted in balsam, the distribution of the iron was more clearly seen. Sometimes in the epithelial cells the blue reaction was a diffuse one with blue granules collected in groups here and there in the cell, in some instances it was found in the inner end of the cell chiefly, while again the protoplasmic processes in the hyaline border gave an intense reaction. Fig. 4 shows some of these details distinctly. In this are represented three cells, in two of which the inner ends appear loaded with iron and they were fixed in the act of transferring it to the underlying tissue. This involves an internal secretion, a process that plays an extensive part in absorption. The iron compound appears to be secreted in a soluble form, for I found that the underlying elements, the connective tissue fibres, yielded frequently a deep homogeneous blue reaction when the lower ends of the epithelial cells gave the same. When the amount of iron in the epithelial cell is small, that part of it in the lower end is dissolved in the protoplasm, but when the amount is large, it appears to be precipitated in a granular form. The dissolved form of the iron compound is possibly an albuminate, but the character of the granular form is difficult to determine, and it is equally difficult to do so in the matter of the deposit in the form of granular masses in the leucocytes below, although on the ground that some of these cells win their way with their iron back into the blood vessels, it is allowable to believe that the iron-holding masses in them, in order not to impede their movements, must be of albuminate composition and not inorganic with fixed shape.

The further course of the absorbed iron it is difficult to demonstrate

unless iron salts are given for some time. It is easy of course to determine that the leucocytes take up some of it. That fact is the one the most prominently seen even when the iron is given in large doses and for a long time, but it is only in the latter case that one is able by micro-chemical means to show that another and probably more important method of transfer exists. When the excised serosa of the upper part of the small intestine from a guinea-pig, fed for some time with either the phosphate, chloride or "peptonate," was treated, after hardening in alcohol, with the acid ferrocyanide mixture and after the usual course of treatment mounted in balsam, the venules appeared blue while the arterioles were unaffected. In all these cases the blue colour was found in both the contents and the intima of the vessels, and where the venule was empty, in the intima alone. In the contents there was a very light blue in the red corpuscles, a white corpuscle here and there contained iron and the plasma was shown to carry it by a colour deeper than that given by the red discs. When the dose was very great indeed the iron in the plasma took a granular form, at least in alcohol preparations, but in this condition the muscular as well as inner coat of the vessel was blue. These venules are radicles of the portal vein. In the liver inorganic iron was found in all the tissues of the peripheral zone of the lobules, in part in a granular form in the peripheral hepatic cells, but diffused mainly through both cell and nucleus of each element. When the dose of iron given was not great, then the iron was mainly if not wholly confined to the peripheral zone. With large doses a greater portion of the lobule was impregnated with iron, and other elements came prominently to view, especially in the "peptonate" preparations. There were leucocytes in the angles of the capillaries in all parts of the lobule but very frequently in the central portion, and their occurrence was manifested under the low power by the strong reaction which they gave for iron (fig. 5). Sometimes each cell was a mass of blue material or it contained large blue masses, in others again, the cytoplasm had a diffuse blue tint with one or more clumps of iron-holding substance. Did these leucocytes come from the villi of the intestine? Some of them undoubtedly must have done so, for, as already stated, in the contents of the venules in the serosa of the upper portion of the small intestine there were a few iron-holding leucocytes. It is probable, however, from a difference in the arrangement and deposition of the iron in them, that a large number have taken up the iron from the plasma after they became entangled in the capillaries. Some of them appear to be so large as to occlude the capillary channels.

What they do with the iron which they contain is a matter of inference only. They probably transfer it to the liver cells through the capillary wall, or, if they again become free, they pass with it on to the general circulation. They are apparently not much discommoded by the amount of iron which they contain, for I found similar iron-holding leucocytes of all shapes and sizes both in the spleen pulp and in the venules leading off from it.

The iron-holding leucocytes of the villi when they leave the latter probably pass into the transverse branches of the capillary network or into the collecting venule of the villus. Outside of the villi the occurrence of iron-holding leucocytes, except in vessels, is extremely rare, although when excessive doses, as, for example, 0·5 grm. daily of ferric phosphate, were given, they were increased in number, owing to the saturation of all the tissues of the intestinal wall with iron.

Whatever iron salt was administered, whether the "peptonate," phosphate, chloride or sulphate, when the dose was small, i.e. under 50 mgrms., the evidence of its absorption was very plain in the villi of the upper end of the small intestine. When the dose was larger, as in the case of the phosphate and "peptonate," its presence was observed in the villi far down in the intestine, but the reaction was the less distinct the more remote the villus examined was from the pylorus. When either of the two was given in very large doses, (0·5 grm. daily of the phosphate for example), the villi near the cœcum gave an intense reaction.

These results can be explained readily. The iron salt of the chyme, when the latter is thoroughly mixed with the biliary and pancreatic fluids, becomes wholly precipitated if the alkalinity of the two latter fluids is sufficiently great. The alkali present may be completely destroyed by a large quantity of iron salt in solution, and when this occurs the excess of the iron salt not precipitated and remaining in solution is absorbed. When the quantity in excess of that necessary to destroy the alkalinity is very great, all the villi of the intestine are in a position to absorb some of it. If on the other hand the dose is small, absorption in the upper end of the intestine is favoured by the circumstance that the three fluids, chyme, bile, and pancreatic juice, do not immediately and intimately mingle and, therefore, the iron is not at once precipitated, some of it being absorbed before that occurs. The quantity of acid in the chyme is a factor of some importance, and when the iron given is in the salt form and not as the oxide, the acidity of the chyme is not decreased, the acid of the salt displaced taking the place of the hydrochloric acid. When the oxide or the reduced metal is

administered, their solution takes up a portion or all of the acid without contributing in turn to the acidity of the chyme, and, therefore, in the intestine the alkalinity of the bile and pancreatic juice goes farther in the precipitation of the salts of iron in solution in the out-poured chyme. The larger the amount of free acid in the latter the greater must be the quantity of iron absorbed.

I have made some experiments also upon the absorption of iron in kittens. One of two from the same litter was given, through a pipette by the mouth, 100 mgrms., in solution, of tartrate of iron and ammonia and both were killed four hours after with chloroform. The villi of the second kitten gave absolutely no reaction for inorganic iron, while in the other fed with the iron salt, the sub-epithelial portions of the tips of the villi gave a marked one. In both the amount of milk in the stomach was the same, the gastric contents in one still holding apparently the greater part of the iron administered.

It was noted in this and in other cases where the dose administered was comparatively small, how free the epithelial cells themselves were from inorganic iron. Although in the sub-epithelial portions of the villi in the kitten the amount of iron was large, the epithelial cells when fixed did not contain the slightest trace of it. The possible explanation is that the cells transfer with great rapidity the iron which they absorb, and that it is only when the cells are fatigued by overwork in this transference, that some of the iron absorbed is seen in them.

I have already referred to the collection of the leucocytes in the tips of the villi and to the invasion of the epithelial layer by leucocytes in the "peptonate" preparations. This is shown in fig. 2, representing an optical section of a villus treated with ammonium sulphide and glycerine. The migration into the epithelial layer does not occur in every villus, while in some exhibiting this appearance it may be more marked than in others. The epithelial cells were very often greatly affected by the invasion, for not only were they considerably displaced, but they were in such a condition of disintegration that in some stained sections the extreme tips of some of the villi appeared denuded of epithelium. Whether the intra-epithelial leucocytes were the cause of this disintegration or not, they contained in addition to iron a great part of the disintegrated material. An invasion of the epithelial layer by leucocytes was also obtained in the villi when a quantity of albuminate of iron, made according to Marfori's[1] method, was given to a guinea-pig. The animal had been fasting for three days when the powdered albuminate, sus-

[1] *Arch. für exper. Path. und Pharm.* Vol. xxix. p. 212, 1891.

pended in water, was given and five hours afterwards it was killed. As this compound is insoluble in weak acids, but readily soluble in weak alkaline solutions, it was not surprising that the villi from the opening of the pancreatic duct to the cœcum gave clear evidence of its absorption, while the villi for half an inch beyond the pyloric valve contained no traces of it. The villi near the distal end of the small intestine contained as much iron, judging by the reaction, as those which were situated near the opening of the pancreatic duct. The number of iron-holding leucocytes was not great, yet they carried a full complement of iron, and in many of the villi a majority of them were intra-epithelial. Their presence in the latter situation was not, as in the "peptonate" preparations, accompanied by a disintegration of epithelial cells, although the latter were frequently much displaced.

These results seem to indicate that combinations of iron and proteid influence in some way the activity of the leucocytes, and, in order to obtain further evidence on this point, observations were made on the lake-lizards (*Necturus lateralis*) in the laboratory. The animals used had been for over thirty months without food, and the intestinal cavities of two examined contained nothing more than small masses of inspissated mucus impregnated with bile, but the mucosa of the same gave preparations of which that represented in fig. 3 is typical. There was iron present in small quantities in the outer ends of the epithelial cells, but it was in the leucocytes that it was most abundant, the cytoplasm of these containing, in addition to what was diffused through it, large granular masses impregnated with iron. Leucocytes giving such a reaction were found scattered all through the mucosa. In the liver and spleen iron-holding leucocytes were found, in the former in the angles of the capillaries and in the latter in the adenoid tissue. To determine if in these animals the leucocytes are affected as they are in guinea-pigs by the albuminate of iron, a quantity of the dried albuminate of iron was dissolved in a very weak solution of bicarbonate of soda and injected through the vent into the intestine of one. Eight hours afterward the animal was killed, the liver, spleen and intestine removed and put into alcohol. The latter organ was only partially opened. In the liver and spleen the amount of iron in the leucocytes was greatly increased and the liver cells gave a deeper diffuse reaction. In the sections from the intestine the leucocytes were found loaded with an excess of iron, so much so that the ammonium sulphide gave them the appearance of huge collections of greenish black granules. In some parts of the intestine they were collected in large numbers under the epithelium

with a few situated in the latter layer, but in other parts, especially near the vent, where naturally the bulk of the injected fluid collected, they were present in very large numbers in the epithelial layer, many of them fixed in all shapes and while migrating with their load of iron compound into the cavity. The best preparations which I obtained, however, were those from the unopened portions of the intestine, for here the contents were retained in the sections. A very large number of leucocytes excessively charged with iron were found in the cavity and others were fixed in the act of passing into it, while in the sub-epithelial leucocytes the iron was abundant. In all the preparations the epithelial cells themselves were comparatively free from the iron compound.

In guinea-pigs fed with the chloride, phosphate, or sulphate of iron, I have not found that the leucocytes were similarly affected, even when these preparations were given without food, nor does the iron present in the ordinary food of the animals produce a like effect, although in all these cases the leucocytes gave unmistakeable evidence of the absorption of these compounds.

It follows from the results of those experiments that inorganic and albuminate compounds of iron are absorbed, at least in the portions of the intestine near the pylorus, and in all parts of the small intestine when the iron compound is not precipitable on mixture with the bile and the pancreatic juice. Undoubtedly also, sulphides in the bowel must remove from solution a quantity of iron in proportion to their abundance. In ordinary diet the extent of the mucosa which absorbs iron must be, in proportion to that which does not, remarkably small. It may possibly be that in the human subject when the iron is specially increased the extent of the absorbing surface is increased, the more so when there is a diminution in the amount of pancreatic and biliary fluids, a condition possible in anæmias. The view held by some that iron salts are not absorbed and that these exercise their effect by stimulating the mucosa to greater physiological activity, is opposed by the results of these experiments. If iron salts stimulate, it is because they penetrate the epithelium of the mucosa and in doing so are transferred to the underlying elements. In other words, they are absorbed. But the extent of the absorbing area is limited; so likewise must be the extent of the area supposedly stimulated and, therefore, the beneficial effects of the stimulation of the mucosa alone must be small. What then is the purpose served by the absorption of iron salts? Leaving out of consideration the possible answer that the iron of such combinations becomes assimilated, that is, is united in the animal cell with other

constituents to form what the histologist calls chromatin, we may discuss some explanations of its effects. The experiments with the feeding of "peptonate" and of albuminate of iron are, of course, too few to enable one to infer anything concerning the action of iron-holding proteids of this character, but all the experiments without exception indicate that the leucocytes have a special affinity for inorganic and albuminate compounds of iron, and it is not too much to infer that this affinity involves a stimulating or chemiotactic effect upon the leucocytes, and that iron salts exercise an effect on other cells corresponding to their function.

The number of leucocytes which are engaged at any one time in the absorption of iron is comparatively small, owing to the small extent of the intestinal mucosa bathed by a solution of an iron salt, and, therefore, the stimulating effect would appear to be small, but it must be remembered that, with the constant stream of iron-holding plasma and iron-holding leucocytes from the villi in the upper portion of the small intestine to the liver, spleen, etc., the other cells of the body, including leucocytes with little or no inorganic iron, are put in position to obtain some of that absorbed. There is also another way in which the question may be viewed. Inorganic compounds of iron, like those of calcium, potassium, and sodium, have been, since the dawn of animal life on the globe, constituents of its media, and of its food, and it is possible that the animal cell has, in acquiring a tolerance for them, accommodated its functions to their presence and has established with them a physiological equilibrium which it may be impossible to maintain in the absence of such compounds. The view that the iron of inorganic combinations goes directly into combination with nuclein and albumin to form chromatins, is one that may in the future be proved correct, but whether it will happen so or not, it is not now, nor apparently will it be then, incompatible with the explanations of other possible functions of iron salts like those just referred to.

In investigating the absorption of iron I had opportunities for determining the mode of excretion, when the iron absorbed is in excess of the needs of the organism. The material illustrating this was obtained from two guinea-pigs, one of which was given a very large quantity of the "peptonate" preparation of iron, while the other received a correspondingly large dose of ferrous sulphate. The secretion of the Lieberkühnian glands of the animal fed with the "peptonate" gave an intense iron reaction, and this was the case not only with the small intestine but also with the cœcum and upper portion of the colon. In the

superficial epithelium of the two latter parts there was no evidence of either absorption or secretion of iron compounds. In the cells of the Lieberkühnian glands no iron reaction could be obtained, nor was it obtained in the same structures in the preparations from the animal fed with ferrous sulphate, although in the latter case the secreted material in the lumina of the glands appeared to consist, in large part, of an iron compound. Possibly the explanation for this is that the cells rapidly transfer with the secretion the iron compound to the lumen and in excessively small quantities at any one moment. The kidneys in both animals gave a very slight diffuse reaction for iron not confined to any part of the organs. It was only in the animal fed with ferrous sulphate that the liver yielded more than ordinary evidence of the excretion of iron. The periphery of the lobules gave an intense iron reaction partly diffused through the hepatic cells, and partly localized in granules situated in that part of each cell bordering on the bile capillary. The latter gave a feeble reaction for iron, especially at the periphery of the lobules. The epithelium of some of the bile ductlets contained free iron, probably absorbed from the bile.

In man the liver and kidney are the most active organs in the excretion of iron. This has been shown by the observations of Hunter[1], Mott[2], and others on cases of pernicious anæmia. In the liver and kidney from a patient, who had died from a complication of troubles in which pernicious anæmia was supposed by some to be a factor, I found this excretion illustrated to a remarkable degree. The bile capillaries were, in some parts of the sections, filled and distended with secreted iron compounds. Where this was the case the cells surrounding a capillary were almost free from inorganic iron compounds, but where the capillary was feebly or not at all injected, then the portion of each cell touching the capillary was loaded with granules of a ferric compound. The sections of the cortical portions of the kidneys gave an exceedingly intense reaction for iron, which, on examination under the microscope, was found chiefly in the convoluted tubules, the cells of these exhibiting both a diffuse and a localized reaction, the latter given by an abundant collection of granules distributed in that half of the cell adjacent to the lumen. The intestine in this case yielded no evidence whatever of the excretion of iron.

The results of experiments on guinea-pigs and of observations on cases of pernicious anæmia show that, in different animals, the organ for

[1] *The Practitioner*, Vol. XLIII. 1889.
[2] *Ibid.* Vol. XLV. 1890.

the excretion of iron may not be the same when this is greatly in excess. This fact has, to a certain extent, been illustrated also in a rutting bitch, in the intestine, liver and kidney of which I could find no evidence of excretion of iron; but sections of its uterus, after being hardened in alcohol, gave in a few seconds a marked reaction for iron confined to the mucosa. Closer examination of these resulted in showing that the iron was deposited in three situations: in leucocytes scattered in large numbers throughout the inter-glandular elements, in the cells of the long convoluted glands, and in the secretion found in the lumina of these. The leucocytes were very much enlarged, the enlargement apparently being due to the brownish masses of iron which they contained. The iron appeared to be in the phosphate form. The cytoplasm gave a reaction also for iron. In the gland cells the iron was both diffused through the cytoplasm and localized in the form of a row of granules immediately adjacent to the lumen of the gland. The diffuse reaction obtained was slight, but that obtained with the granules was an intense one, and the arrangement of the granules seemed to suggest that they were caused by precipitation, in an inert portion of the cell, of iron which had been in solution in the more active parts of the cytoplasm. The substance in the lumen of each gland gave a weak, but still distinct reaction for iron. Whether the leucocytes in the inter-glandular tissue carried the iron to the mucosa from other parts, or obtained it by absorption from the lymph bathing them, it was not possible to determine definitely. I could find no leucocytes in the blood vessels which contained inorganic iron compounds, and this, considered in connection with the large size of the iron masses in the inter-glandular cells, suggests that the latter derived their iron from the lymph of the inter-glandular tissue. In this case the lymph must have been also the source of the iron in the gland cells.

Why different organs in different animals should serve for the excretion of a great excess of iron, it is difficult to say. Possibly this depends on differences in the degree of activity which the organs present in different animals. It is quite as difficult to say why the kidney in some cases of pernicious anæmia and not in others should give such marked evidence, in the convoluted tubules, of the excretion of iron.

II. The Absorption of Organic Iron Compounds.

The expression "organic iron compounds" includes two chief classes of substances:—

1. The assimilated compounds of iron; that is, nuclein and related compounds, including Bunge's hæmatogen. These may be conveniently termed the "chromatins."

2. Compounds produced by a degenerative process from the first class. This includes hæmoglobin, melanin, and lardacein.

I have limited my investigation to representations of the chromatin class.

Bunge believes that hæmatogens are formed only in vegetable life, and constitute the only compounds of iron which are absorbed by animals.

Feeding experiments with these substances have been made by Socin[1] and Busch[2]. In one experiment Socin found the amount of ingested iron exceeded that in the excreta. In the other two experiments the reverse was the case. These experiments were made on dogs. With mice he found that iron-free food mixed with organic or inorganic iron compounds did not sustain life any longer than food free from iron, whereas control animals fed on a more natural food (coagulated egg-yolk) lived healthily. Busch from analyses of the iron in the urine after feeding on hæmatogen, hæmoglobin and hæmatin, concludes that of these hæmatogen is least absorbable. Bokay[3] found that nuclein is not digested by artificial pancreatic juice, that it leaves the body chiefly in the fæces, and causes but a slight increase in the urinary phosphates[4].

I began my own observation by feeding lake-lizards (*Necturus lateralis*) on chromatin isolated by artificial gastric digestion from lambs' testicles. The lizards were killed, and the intestines investigated by the micro-chemical methods already described. The results were negative. I then fed mice on chromatins mixed with starch and lard; they died in from six to fifteen days with symptoms of diarrhœa. They also died in a few days when the chromatin was mixed with coagulated

[1] *Zeitsch. f. physiol. Chem.* Vol. xv. p. 93, 1891.

[2] Kobert. *Loc. cit.*

[3] *Zeitsch. f. physiol. Chem.* Vol. I. p. 157, 1877—8.

[4] In Gumlich's experiments, a description of which was recently published (*Zeit. für physiol. Chemie*, Vol. XVIII., parts 5 and 6, p. 508, 1894), the absorption of nucleinic acid in the dog appeared to be shown by the increase in the amount of phosphorus excreted in the urine after the administration of a quantity of compound.—P. M. Popoff (*ibid.* p. 533) found that nuclein compounds enter into solution only in very small quantities in gastric digestion but in greater quantities as the result of the action of artificial pancreatic juice, and he points out that the occurrence of such compounds in a dissolved form is all that is necessary for their absorption in the intestinal tract.

egg-yolk, but thrived well on the egg-yolk without such admixture. I also gave to a lake-lizard the fluid from an artificial pancreatic digestion of nuclein; this fluid, however, contained little if any nuclein in solution, and micro-chemical investigation of the lizard's intestine gave negative results.

The failure of all these experiments led me to use a less abnormal kind of food; and, since, according to Miescher[1], egg-yolk itself contains 1 to 1·5 per cent of nuclein (hæmatogen), that food substance appeared likely to yield the best results.

I used unboiled egg-yolk, for when egg-yolk is hard-boiled the yolk spherules become thereby fixed in form, and the chromatin-holding particles are set free only when the spherules are digested, but when the yolk is administered fresh the spherules readily undergo fragmentation and the chromatin-holding particles are liberated and put in a form in which the epithelial cells, if they possess the power, can invaginate them. In the spherules the chromatin is partly in a granular form[2] and, apparently, partly as envelope material to its fat globules, the latter varying in size and shading off into the small granules in such a way as to suggest that the latter are also fat globules of almost infinitesimal size surrounded by chromatin. Fig. 14 gives a representation of two yolk spherules which were fixed by heat and in which the iron, set free by sulphuric acid alcohol, was converted by ammonium sulphide into the sulphide. In it can be seen smaller and larger fat globules surrounded by an iron-holding envelope. The fat is, therefore, closely associated with the chromatin, and as we know the former is in some way absorbed by the intestinal epithelium, the conclusion did not appear to be a strained one that both constituents are absorbed together.

The first experiments were made with mice. The animals were isolated and fed for periods ranging from five to fifteen days with fresh egg-yolk and preparations of the small intestine were made in various ways to determine the effect. Owing to the liquid or semi-liquid form of the food the animals partook but slightly of it, and consequently there were found in the intestinal epithelium no very marked indications of absorption, even of fat. In a series of preparations from one of the animals which was allowed to live for the longer period, I found that, in the internal or lower half of each epithelial cell, the protoplasm

[1] Miescher. *Hoppe-Seyler's med. chem. Unters.* Pt. 4, p. 502, 1871.

[2] Miescher (*loc. cit.*) localised the nuclein which he discovered in egg-yolk in the granules of the yellow yolk spherules.

possessed a greater capacity for staining matters like eosin than is exercised by the same cells ordinarily and, further, that this same protoplasm was denser and more finely granular. Owing to the smallness of the sections, manipulation with acid alcohols to set free the masked iron[1] to determine if this staining capacity was due to chromatin, did not meet with much success, although the teased-out cells after hardening in alcohol and long treatment with warm ammonium hydrogen sulphide and glycerine gave results which seemed to support that view. These experiments, however, indicated the direction in which my succeeding observations were to be conducted.

A large number of guinea-pigs were then fed for different periods with undiluted yolk in excess. Each animal was given about 10 c.c., three times a day, administered by the mouth by means of a glass pipette, and when it was killed the small intestine was removed and hardened, one part in alcohol and another in corrosive sublimate. The sections from the sublimate preparations made with paraffin and fixed on the cover glass by Gaule's method, were either stained with hæmatoxylin and eosin, or put through nitric acid alcohol for 8—10 hours at 35°C. and then treated with the acid ferrocyanide mixture to determine the distribution of "masked" iron. The alcohol material was also treated in the same way, with this exception, that the sections were fastened to the cover slip with collodion before the paraffin was removed, and when the sections were made by the celloidin process they were passed directly through nitric or sulphuric acid alcohol. The results of these experiments and methods were very interesting.

In the guinea-pig, as ordinarily fed, the "masked" iron exhibits in its distribution in the epithelium of the intestine very little difference from that represented in fig. 6, in which the iron is shown in the chromatin of the nuclei and in a narrow zone immediately about some of the nuclei, but in preparations from animals fed with yolk for two or three days, the epithelial cells situated on the sides of the villi and below the tips of the same have the iron distributed as represented in fig. 7, in which the whole of the protoplasm in the lower half of each cell and in the leucocytes below give a uniformly diffuse Prussian blue reaction. The epithelial cells at the tips of the villi are so much distorted by the fat present in them, that a division of each into an internal and external part is impossible, except in some cases where the absorption of fat has ceased to take place. In preparations stained

[1] The method here referred to will be described at full length, in a forthcoming paper on the distribution of assimilated iron in animal and vegetable cells.

with hæmatoxylin and eosin, the cells immediately below the tip give usually the appearance represented in fig. 8, but with this exception, that the bodies enclosed in cavities of the protoplasm in the external half of each cell shown in the figure are not present in the preparations from all the animals fed with egg-yolk. The internal half of each cell is loaded with finely granular, eosinophilous material in which vacuoles are discernible, the outer half having its protoplasm arranged in a coarse meshwork, the cavities of which were probably occupied by fat globules. The nuclei in the majority of these cells contain an eosinophilous substance filling the spaces left by the chromatin network. In some villi the absorption of fat had occurred to such an extent that the epithelium was separated from the underlying tissue, as represented in the figure, and in these cases one sees sometimes a condition which explains the destiny of the finely granular, eosinophilous material in the inner ends of the cells. The corrosive sublimate has coagulated a proteid material extending from each cell and containing some eosinophilous granules. This condition I regard as due to *internal secretion* on the part of the epithelial cells, and it has its parallel in the same epithelial cells when they are transferring iron salts from themselves to the internal elements, a process which I have already described. There is a difference, however, in the two in one respect, a difference which may only be apparent; in the internal secretion of iron, the latter appears to become dissolved before passing from the epithelial cell internally. In the yolk preparations, the eosinophilous material is in a granular form, and could one be certain that it was not due to irregular precipitation by the corrosive sublimate, it would be an indication that the epithelial cell secretes internally matters in a solid or semi-solid form. The irregular form taken by the secreted matter may be due to conditions governing the diffusion of the hardening reagent, but, from the way in which each shred of the hardened proteid secretion is arranged in its attachment to an epithelial cell, one is disposed to regard it as fixed in the condition which it occupied in the living tissue. Whether this secretion contains "masked" iron or chromatin I cannot say.

Now as in the secreting cells of all sorts, and especially in those of the Lieberkühnian glands, secretory activity is associated with the presence of a chromatin in the part of the cell remote from the lumen (fig. 9), it might be urged that the increase of the "masked" iron in the inner ends of the superficial epithelial cells of a villus was due not to an iron compound absorbed, but to secretory activity bringing about

an increase of the substance governing that process. This objection has some force, for so constantly is the presence of a chromatin (prozymogen) connected with the processes of secretion, especially in those glands which furnish a ferment, that one hesitates to deny the existence of a prozymogen in another phase of cell activity but little, if at all, different from that of secretion. The only evidence on which one can rely to show that some of the chromatin of the yolk was absorbed in this case is, that the iron reaction of the liver from an animal fed for some time with yolk is of a different character from that given by the liver of the animal after its ordinary diet. In two guinea-pigs, one fed for seven days, the other for six, when it died in a comatose condition, there was undoubted evidence of the absorption of the chromatin, for, in sections of the livers hardened in alcohol, the application of warm ammonium hydrogen sulphide for some hours gave a reaction which was most marked at the periphery of each lobule, but present also in the central zone of the same. The reaction in the central portion was more difficult to get and was best obtained when the sections were very thin. In these preparations one can determine that the iron reaction gradually becomes less intense as one follows the tissues of the lobules from the periphery to the centre. In the liver of the animal after ordinary diet the reaction with ammonium hydrogen sulphide is obtained only in the outer zone and faintly in the outer portion of the middle zone. In these, further, iron in a coarsely granular form is almost always found in the cells at the periphery of the lobule, but such granules are absent in preparations from the animals fed with yolk for some time. In the latter the iron reaction in the liver cell is a diffuse one and it is present in the leucocytes of the capillaries as well. What seems to show that this reaction is not due to inorganic or albuminate iron, but to that in the organic form, is the length of time required to bring it out. These results have been obtained also in experiments on *Amblystomata.*

In *Amblystoma* the cellular elements, although not of the size of those in *Necturus,* are very large, and as it was possible to obtain these animals in numbers sufficient for experiments in this line, I used them for this purpose. The yolk was given through a glass pipette, introduced through the mouth into the stomach, and in quantities corresponding to the size of the animal. Owing to the ease with which they may be fed, and because of the retention on the part of the stomach of what is so given, these animals are very suitable objects for experiments of this sort. Now, in those recently captured or kept

in captivity for a week or so without being fed, there is comparatively little inorganic iron in the liver, no more, in fact, than there is in the liver of *Necturus* after long captivity, and in sections of the liver of such, when hardened in alcohol and treated with nitric acid alcohol, or sulphuric acid alcohol, to set free the "masked" iron, the latter is wholly confined to the nuclei of the cells. When, however, yolk is given daily in such quantities as are retained to these animals for a period of four days or more, the liver cells, which are loaded with fat, present a different reaction. When hardened in alcohol, sections of the liver give, after treatment with sulphuric acid alcohol or nitric acid alcohol for sixty hours, a reaction for iron like that shown in fig. 13. The cytoplasm of the hepatic cell, arranged in a coarse meshwork enclosing the fat droplets, carries in its trabeculæ irregularly shaped masses of iron-holding substance which are sometimes extended along and in the trabeculæ. The nucleus gives the usual reaction for "masked" iron. The iron in the cytoplasm is of the "masked" character, for isolated cells mounted with a mixture of ammonium hydrogen sulphide and glycerine, and kept at a temperature of 60° C., gave an iron reaction in the masses in the cytoplasmic trabeculæ only after three days, which became more marked on the fifth day. The nuclei in these cells gave but a feeble reaction for iron owing, apparently, to the fact that the "masked" iron in the cytoplasm had used up all the decomposing energy of the sulphide and therefore, apparently, little of the active reagent reached the nuclei. That the iron in the masses of the cytoplasm is not due to the diffusion from the nucleus, was shown by the non-occurrence of such iron compounds in the cytoplasm of hepatic cells from *Amblystomata* recently captured, or kept in captivity without food. In those *Amblystomata,* in the intestine of which on capture, food matters, such as mollusca, worms and insects, were found, the cytoplasm of the hepatic cells gave no reaction for "masked" iron. The difference in this respect between the liver cells of the recently captured or fasting animal, and those of the animal which had been fed for several days with egg-yolk, was readily shown by putting thin sections of the organs, hardened in alcohol, taken from animals in the two conditions, into the same dish of sulphuric acid alcohol for three days. At the end of this time treatment of the two kinds of sections with the acid ferrocyanide mixture gave different results in both cases. In both the nuclei exhibited an equally intense iron reaction, but in the cytoplasm of the cells from the fed animals, the masses of the trabeculæ were as distinctly iron-holding as in the

cells treated with ammonium hydrogen sulphide, while the cells from the livers of the other animals exhibited no such reaction.

This subjection of sections to treatment under exactly the same conditions, considered in connection with the results obtained by using ammonium hydrogen sulphide, shows that the iron found in the masses situated in the cytoplasmic trabeculæ was due to a "masked" combination there placed. That it was a chromatin was shown by its capacity for taking up staining matters. In sections of the liver in this case, when hardened either by the alcohol or corrosive sublimate method and stained with hæmatoxylin, safranin, or eosin, the cytoplasmic masses were coloured, though not as deeply and intensely as the nuclear elements, yet as distinctly as the latter. In sections from the liver of the unfed or recently captured animal, when similarly treated, there was a complete absence of such stainable material in the cytoplasm. When sections of the liver from the fed animal were treated with sulphuric acid alcohol to set the "masked" iron free and subjected to the Prussian blue reaction, subsequent staining with safranin gave a violet-like combination of the blue and the safranin red in the chromatin of both the cell and the nucleus.

That the cytoplasmic chromatin in this case comes from the chromatin of the yolk, and is not due to a prozymogen called into existence by the abnormal quantity of food matter in the hepatic cell, I tried to show by feeding *Amblystomata* with olive oil. In those fed with yolk the hepatic cells were very greatly loaded with fat. A similar excess of fat in the liver cells was obtained by feeding with oil, but in these there was no cytoplasmic chromatin.

In thin sections from the intestine of the *Amblystoma* fed on egg-yolk, the presence of "masked" iron compounds in the cytoplasm of the epithelial cells was not indicated readily, owing to the abundance of fat in the same. The more active the absorption of the yolk elements had been, the less, therefore, was it possible to demonstrate the existence of cytoplasmic chromatin. I have found, however, as in the intestinal epithelium of guinea-pigs after they are fed on yolk for some time, that the inner portion of each epithelial cell not overloaded with fat, was filled with an eosinophilous, finely granular material from which sulphuric acid alcohol sets free iron. I found also that if an *Amblystoma* is fed for several days with yolk and then allowed to fast for several days, in its intestinal epithelium now freed from fat there is, as shown by sulphuric acid alcohol, a slight amount of "masked" iron diffused through the cytoplasm. Whether this is derived from the yolk, or is

that of a prozymogen, it is as difficult to decide as in the case of the intestinal cells of the guinea-pig under similar conditions.

There is a difference between the chromatin of the yolk and that of the hepatic cytoplasm of an *Amblystoma* fed with yolk, which is probably to be explained as due to a chemical transformation effected by the absorbing cells or by the liver cells. According to Bunge[1] the chromatin of egg-yolk, or hæmatogen, as he terms it, when isolated, gives no immediate reaction with ammonium sulphide, but after the lapse of half-an-hour, a slight green colour appears which, in the course of several hours, deepens to an intense dark green, and several days after becomes black and opaque, the various appearances being hastened by increasing the volume of ammonium sulphide added. I find that the addition of ammonium hydrogen sulphide to fresh yolk, calls forth in two or three minutes at most the fullest iron reaction of which it is capable. The difference in this respect between isolated and unisolated yolk chromatin, is to be explained by the finer division and distribution of the latter in the spherules which the reagent disintegrates and partially dissolves before attacking the chromatin, and by the consequently readier action of the sulphide. When, on the other hand, the spherules have been fixed by heat or by alcohol before subjection to ammonium hydrogen sulphide, the iron reaction is almost as slowly obtained as in the case of the isolated yolk chromatin, the slowness of the reaction being partly caused by the difficulty with which the sulphide penetrates the spherules. In the livers of *Amblystomata* fed with yolk the cytoplasmic chromatin is readily reached by the ammonium hydrogen sulphide, but it takes several days to give the iron reaction, this indicating that the iron there is more firmly held than it is in the yolk. This difference corresponds to that found in the yolk as it is being assimilated in the developing larvæ of *Amblystoma*. In these the yolk chromatin is in form of homogeneous spherules which, when fresh or hardened in alcohol, give, in a minute or two after treatment with ammonium hydrogen sulphide, a dark green reaction for iron, also shown, though less readily, by the nuclei of the cells containing the spherules. Until the spherules are completely dissolved by the containing cells the nuclei give this reaction, but when they have disappeared then the iron reaction in the nuclei is obtained with as great a difficulty as it is in those of the adult animals. In other words, the yolk chromatin, as it is assimilated, undergoes a change whereby

[1] *Loc. cit.* I have elsewhere criticised Bunge's description of this compound and have pointed out some mistakes into which he has fallen.

the iron in it is more firmly fixed or more "masked." It is extremely probable that the difference between the chromatin of hen's egg and that of the hepatic cytoplasm of *Amblystomata* fed with it, is to be explained in the same way[1].

The yolk of old or stale eggs gives, with ammonium sulphide, the iron reaction almost immediately, and is, in this respect, more suitable than the fresh yolk to determine if the absorption of yolk chromatin obtains. I made two experiments with it, one with a guinea-pig kept without food for seven days, the other with a lake-lizard which had been kept without food for over two years.

The guinea-pig was killed five hours after it was given 12 c.c. of stale yolk, and parts of the small intestine and the liver were removed and put into alcohol. Pieces of both organs were, in the fresh condition, teased out on the slide in a drop of ammonium hydrogen sulphide and immediately examined under the microscope. The preparations from the liver yielded nothing distinctly demonstrating that absorption had taken place, but in the tips of the villi in which fat absorption was prominently shown, there was a dull green colour apparently confined to the sub-epithelial elements. The fat-holding epithelial cells of the fresh villi were easily removed in some instances by teasing, and in some of these denuded villi appearances were obtained with ammonium hydrogen sulphide which resembled that represented in fig. 10. Here greenish granules were found in the adenoid tissue and the leucocytes which, in some instances, were collected in groups immediately under the epithelial layer. Halfway down the villus the greenish reaction became indistinct. In sections of the villi hardened in alcohol, the ammonium hydrogen sulphide gave a green reaction most frequently confined to a zone immediately below the epithelium of the tips of the villi, but sometimes extending into the lower ends of the epithelial cells themselves. The reaction of the sub-epithelial zone was chiefly in the leucocytes, and in these the iron was rarely in the

[1] Some change takes place in the iron-holding substance in the cytoplasm of the hepatic cell in *Amblystoma*, as shown by its staining reactions. In the animals fed with egg-yolk for some time, the hepatic cells, when hardened with alcohol or corrosive sublimate and stained with hæmatoxylin and eosin, were found to contain, in that part next the blood capillary, eosinophilous masses and in that part adjacent to the bile capillary masses which had a violet stain. Both species of contents were formed of an organic iron compound and between both there were transition elements in which the affinity for hæmatoxylin was as great as that for eosin. It would appear as if the eosinophilous substance which, judging from its position, is possibly derived by the cell from the blood, becomes converted by the cell into that substance which manifests a greater affinity for hæmatoxylin.

granular form observed in the fasting animal or in one fed on ordinary diet. This difference indicates that the iron in these cases is derived from different sources. The difference between the disposition of the iron in the fresh villus, and that found in the villus hardened in alcohol, is to be attributed to the ammonium hydrogen sulphide altering the arrangement and character of the sub-epithelial elements when these were fresh, and to the extraction by the alcohol of the fat droplets and globules in the leucocytes which, consequently, were more uniformly coloured by the reagent than was the case with the fresh elements.

There was evidence of the transference of yolk chromatin from the epithelial cells to the underlying elements, and this was found in those villi in which the process of fat absorption had not distorted the cells. Sections of these villi, obtained from material hardened in alcohol, when treated with ammonium hydrogen sulphide, gave preparations like that of which fig. 12 is an illustration. The inner portions of some of the cells at the extreme tip of a villus gave a faint greenish reaction immediately after the reagent was added, but in the corresponding portions of other epithelial cells the reaction was given also by granular elements lying between and among the fat droplets. When the cell was loaded with fat the greenish reaction was not obtainable. The underlying leucocytes appeared, frequently, placed against the lower ends of the epithelial cells, and when this was the case the greenish granules in the latter were accompanied by similar elements in the more superficially placed leucocytes. The iron compound in the lower portions of the epithelial cells differed, in the readiness with which it reacts on the addition of the sulphide, from that found in the same structures when fresh yolk was fed, as in the earlier experiments.

The lake-lizard, five hours after 4 c.c. of stale yolk was injected into its intestine, was killed and the intestine removed, cut open and put into alcohol. Sections, as well as teased-out portions of the hardened mucosa, were examined when mounted in a mixture of the sulphide and glycerine, both before and after the application of warmth. The results of this experiment were not as decisive as those of the last described, for the reason, apparently, that as the digestive and absorptive powers had been so long unused, a longer time than five hours for the absorption of the yolk should have been allowed. The yolk had mingled with mucus and in many places formed with it a coagulum which, even in the fresh state, came away leaving the surface of the mucosa perfectly clean. In other parts, chiefly at the tips of the

longitudinal folds, fat absorption had occurred, causing an extraordinary distortion of the epithelial cells and amongst these, shaped and moulded by the fat-loaded cells, were leucocytes which also held fat and at the same time gave a very strong iron reaction with ammonium hydrogen sulphide. Below these points there were large collections of leucocytes with scarcely less iron than had those amongst the fat-holding epithelial cells. Leucocytes were also found in the tips of other folds projecting beyond the free border between fat-holding epithelial cells, the part projecting being surrounded by yolk and containing both fat and iron compounds. That a portion of this iron was of the organic form was shown by the fact that the reaction first obtained with ammonium hydrogen sulphide became deeper when the preparation was kept for an hour at 60° C. It of course does not follow that this was derived from the yolk, but that they received a part of it from this source, appeared to be indicated by the greater abundance of iron-holding leucocytes under those tips of which the epithelial cells were loaded with fat.

How the chromatin is transferred to the liver I cannot say definitely, but the capacity of the leucocytes for carrying inorganic compounds of iron and the occurrence of iron-holding leucocytes in the capillaries of the liver of an *Amblystoma* fed for a time with yolk, gives a basis for the opinion that they are the chromatin carriers. Furthermore, we know that in the performance of their function as phagocytes they invaginate and dissolve the remains of disintegrated cells, and there is very little apparent difference between this and that of taking up particles, holding chromatin, which may reach them through the process of internal secretion already described. This view would explain the occurrence of leucocytes which gave the diffuse iron reaction in the villi of guinea-pigs fed with stale yolk.

There remains another question: How does the chromatin of the yolk gain access to the interior of the epithelial cells? Upon this point I have not much to offer, although I have often endeavoured to determine the mode of entrance. The intimate relations of the fat and the chromatin in the yolk suggest that the method of absorption of both is the same, that is, that when the epithelial cell takes in fat, it also receives the chromatin which appears to form envelopes for the most minute fat particles in the yellow yolk spherule. The chromatin of yolk would, when deprived of its intimate association with fat, therefore, be unabsorbable, even though mechanically mixed with a quantity of the same. This would explain some of the results of

Socin's experiments already quoted. That investigator fed mice with pure hæmatogen (of yolk) mixed with the proteids of serum, hog's fat, and starch, and found that the animals did not live as long as those fed on absolutely iron-free food, while those fed on hard-boiled egg-yolk mixed with starch and cellulose lived and throve on the diet. If the fat of yolk assists in the absorption of its chromatin, then yolk from which the fat has been extracted with ether, mixed with hog's fat and starch, ought to give the same result when fed to mice as was obtained in Socin's experiments with pure hæmatogen mixed with other food elements. The result of such an experiment would indeed be interesting.

As already mentioned, I have made frequent attempts to determine how the particles of chromatin enter the epithelial cells. I have in all of these used alcohol material, which was teased out on the slide and mounted in glycerine and ammonium hydrogen sulphide. The idea with which I made these attempts was that the chromatin particles, rendered greenish by their iron reaction, ought to be easily seen with careful adjustment of light and the best optical appliances obtainable. Of all these endeavours but one seemed to give good results. This was furnished by the epithelium of an *Amblystoma* which had, 24 hours before being killed, been artificially fed with yolk. The appearances to be described were found in isolated spots only on the tips of the longitudinal folds in the intestine. The free margin of the cell was covered with granular matter from the yolk, in which one could see very minute fat globules intermingled with particles having a greenish colour. The line of separation of this material from the striated border was, in the majority of the few cases observed, not distinct. The striated border did not give the usual appearance, the striae being apparently replaced, each by a row of oval vesicles with greenish envelopes. With 1·5 mm. apochromatic immersion (Zeiss) and compensation ocular 4, the vesicles could be seen connected by a grayish line. On the protoplasmic side of the margin were also minute vesicles with greenish envelopes, apparently of the same character as those in the striated border and the reticulated protoplasm itself had a slightly greenish tinge (fig. 15). Whether the oval vesicles in the margin were in the rodlets (Stäbchen of the Germans) or between the same it is not possible to say, for in this case one deals with structures which, in their size, and optical properties, approach the limits of microscopic vision. It was also impossible to determine whether these vesicles were being invaginated by the epithelial cells or were mechanically entangled by

the rodlets. It may, in fact, be that the oval vesicles were not connected with absorption at all and that they were merely appearances in the rodlets, although, on this view, the green reaction in their envelopes would be difficult to explain. That the rodlets are not always simple structureless elements, I have, several times, found to be the case in preparations from the guinea-pig (fig. 11), in which each rodlet appeared to be a series of beadlets or granules. These may have been produced by the reagent used, but their occurrence indicates that the rodlets are not homogeneous elements. Heidenhain[1] observed in the salamander, the dog and the cat, a thickening of the lower end of each rodlet. In my preparations the basal granule of each rodlet was most distinct.

It does not appear that the absorption of chromatin and fat goes on in the proportion in which they occur in yolk. In sections, made by the celloidin method, of the intestinal mucosa and its adherent yolk, from an *Amblystoma* fed with yolk, I found above and resting upon the epithelium, here and there, homogeneous clumps of chromatin which are not found in the yolk in its ordinary condition. When these sections are for some time kept in contact with ammonium hydrogen sulphide at 60° C., the clumps give a decided iron reaction. The occurrence may be explained by the existence, on the part of the epithelial cell, of a capacity for the absorption of fat greater than that for the absorption of chromatin.

I have already referred to the occurrence of bodies in the outer halves of the intestinal epithelial cells in preparations from a guinea-pig which had been fed with yolk for thirty-six hours. These elements are not iron-holding, as they do not yield any iron reaction after treatment for days with ammonium hydrogen sulphide, or after treatment with acid alcohols. They possess, however, a greater affinity for dyes like eosin and aurantia than the surrounding cytoplasm, and solutions of Ehrlich's hæmatoxylin give them a feeble violet colour. Like nuclear chromatin they absorb and tenaciously hold mineral reagents, as, for example, ammonium molybdate[2] and this fact, considered in connection with their capacity for absorbing dyes, would seem to suggest that they belong to the nuclein class of compounds. Somewhat similar bodies have been observed by Heidenhain[3] in the epithelial cells of the intestinal villi in

[1] *Archiv für d. ges. Physiol.* Vol. LXIII. Supplement, 1888.

[2] As employed by Lilienfeld and Monti to determine micro-chemically the presence of phosphorus (*Zeit. für physiol. Chemie.* Vol. XVII. p. 410).

[3] *Loc. cit.*

a new-born pup which had suckled, and he has figures also, in one of the plates accompanying his paper, of a portion of a villus from a rabbit after being fed with milk, in the epithelial cells of which are shown a number of similar bodies. He found their size and number to vary in different cells, while they were absent in the fœtus and in a pup twelve days old. He regarded them as albuminous excretions from the protoplasm which appear at the commencement of the absorptive process, but gradually vanish. If the bodies which I find in my preparations are of the same nature as those described by Heidenhain, a different explanation of their origin appears necessary. It is remarkable that they should occur after the commencement of milk or yolk feeding, and as both foods contain proteids intimately associated with the fat globules, the absorption of the proteids, as well as of the fats, must entail upon the cells the disposal of some of the former which are at first physiologically cumbersome as it were, and which, possibly, are disposed of temporarily in the form of these protoplasmic masses. The casein of milk and the vitellin of yolk, both phosphorus-holding compounds, may thus contribute to their formation. The only difficulty experienced in accepting this view of their origin is that it does not explain, in the results obtained with a yolk diet, why these elements are absent where the yolk absorption goes on most vigorously, that is, in the cells at the extreme tips of the villi, while they are present in those cells in which yolk absorption is less active, and why also these elements are absent after two days of a yolk diet. Further investigation is necessary upon all these points.

Summary.

1. The experiments on the administration of inorganic compounds of iron to guinea-pigs and other animals have resulted in showing that the intestinal mucosa absorbs these to an extent which varies with the nature of the compound and with the quantity of it given. When the dose is small, absorption occurs only in that part of the intestine adjacent to the pylorus and measuring only a few inches in length, yet when the quantity given at any one time is large, the absorptive area may embrace the whole of the small intestine. In the former case the result appears to depend on the complete precipitation, as hydroxide, of the iron of the salt unabsorbed, in the thoroughly mixed bile, chyme, and pancreatic juice; and in the latter case the large amount of the iron salt, apparently, first destroys the alkalinity of these fluids, the excess

of the salt unaffected and remaining in solution then undergoing absorption.

2. The intestinal epithelial cells transfer the absorbed iron at once to the underlying elements when the quantity absorbed is small, but with a large amount absorbed the epithelial cells are found to contain some of it.

3. Though some of the sub-epithelial leucocytes of the villi appear to carry part of the absorbed iron into the general blood circulation, probably the more important agent in the transference of the inorganic iron from the villi to other parts of the body is the blood-plasma.

4. Marfori's albuminate and the commercial "peptonate" of iron, when administered to guinea-pigs, seem to stimulate the leucocytes to invade the epithelial layer of the intestinal villi.

5. Of the organic iron compounds belonging to the "chromatin" class, that present in egg-yolk (hæmatogen of Bunge) undergoes absorption in the intestine of the guinea-pig and of *Amblystoma*. In these, but more especially in the latter, after they are fed with egg-yolk for several days, the cytoplasm of the liver cells yields marked evidence of the presence of an organic iron compound belonging to the "chromatin" class and derived from the yolk fed.

6. The mode of absorption of yolk "chromatin" is obscure, but the process appears, in some way, to be connected with the absorption of the fat with which the iron compound is closely associated in yolk.

EXPLANATION OF FIGURES.

PLATE XI.

Note. In the preparation of all the figures (except 15) Abbe's camera was employed and all, with the exception of two, are illustrated as they were seen with an immersion apochromatic objective (Zeiss 3 mm., 2 mm. or 1·5 mm. focus). The exceptions are Figs. 1 and 2, in the drawing of which Zeiss D. was used.

Fig. 1. Section of a villus from the pyloric end of the small intestine of a guinea-pig kept on ordinary diet. Alcohol, acid ferrocyanide mixture, balsam. × 305.

Fig. 2. Optical section of a slightly compressed villus from a guinea-pig after the administration of "peptonate" of iron. *l*, the lacteal vessel. Alcohol, ammonium sulphide, glycerine. × 305.

Fig. 3. A portion of the mucosa of the intestine in a lake-lizard. *e*, epithelial cells, *l*, iron-carrying leucocytes, *r*, red blood corpuscles, also shown to contain inorganic iron. Alcohol, acid ferrocyanide mixture, balsam. × 620.

Fig. 4. A portion of the epithelium and underlying elements of an intestinal villus of a guinea-pig after the administration of "peptonate" of iron. *l*, leucocyte, *bc*, blood capillary. Alcohol, acid ferrocyanide mixture, balsam. × 1240. Drawn with the diaphragm of Abbe's condenser removed from the microscope.

Fig. 5. Portion of a section of the liver of a guinea-pig fed with "peptonate" of iron. *l*, leucocytes, *hc*, hepatic cells, *bc*, blood capillary. × 1240. Drawn with the diaphragm of the condenser removed.

Fig. 6. A portion of the tip of an intestinal villus of a guinea-pig kept on its ordinary diet, to show the distribution in the cells of the organic iron compounds (chromatins). *e*, epithelial cells, *l*, leucocytes, *a*, nuclei of adenoid elements. In the cytoplasm of two of the leucocytes are found granules of an inorganic (?) iron compound. Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. × 1240. Drawn with the diaphragm of the condenser removed.

Fig. 7. Epithelium and underlying elements from the side of a villus of a guinea-pig on the second day of the course of yolk-feeding, to show the distribution of organic iron compounds (chromatins). *l*, leucocytes, *a*, the sub-epithelial "membrane." Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. × 1240. Drawn with the diaphragm of the condenser removed.

Fig. 8. Epithelium and underlying elements of a villus of the same animal. *a*, sub-epithelial "membrane," *s*, the secretion from epithelial cells. Corrosive sublimate, hæmatoxylin, eosin, balsam. × 1240.

Fig. 9. Portion of a Lieberkühnian gland of a guinea-pig to show the distribution of organic compounds of iron (chromatins) and especially of those connected with secretion. Alcohol, nitric acid alcohol, acid ferrocyanide mixture, balsam. × 620. Drawn with the diaphragm of the condenser removed.

Fig. 10. Portion of a fresh villus of a guinea-pig killed five hours after being fed with stale yolk. The epithelium has been removed. Ammonium hydrogen sulphide, glycerine. × 620.

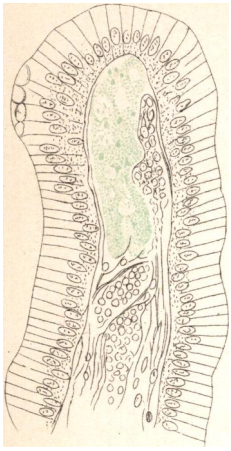
Fig. 11. Intestinal epithelial cell of a villus from the same animal. Alcohol, ammonium hydrogen sulphide, glycerine. × 820. (Zeiss oc. 4, apochr. imm. 1·5 mm.)

Fig. 12. Epithelium and underlying leucocytes of a villus from the same animal, to show the absorption of the yolk chromatin. Alcohol, ammonium hydrogen sulphide, glycerine. × 620.

Fig. 13. A liver cell of an *Amblystoma* fed artificially for four days with egg-yolk. Alcohol, sulphuric acid alcohol, acid ferrocyanide mixture, balsam. × 820. Drawn with the diaphragm of the condenser removed.

Fig. 14. Yolk spherules from a hard-boiled egg, to show the distribution of the iron (of the hæmatogen). In the spherule on the left the elements are fewer and coarser. Sulphuric acid alcohol (for 48 hrs.), ammonium hydrogen sulphide, glycerine. × 820. (Comp. oc. 4, imm. apochr. 1·5 mm.)

Fig. 15. Free border of an intestinal epithelial cell of an *Amblystoma*, fed with yolk. *p*, cell protoplasm, *h*, hyaline border, *y*, elements of yolk. Alcohol, ammonium hydrogen sulphide and glycerine (at 60° C. for eight hours). (Comp. oc. 4, imm. apochr. 1·5 mm.)



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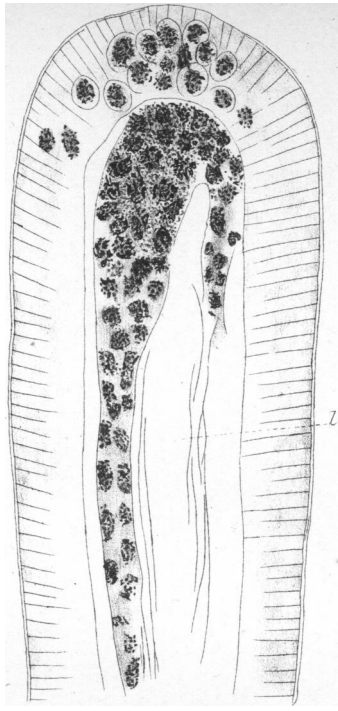

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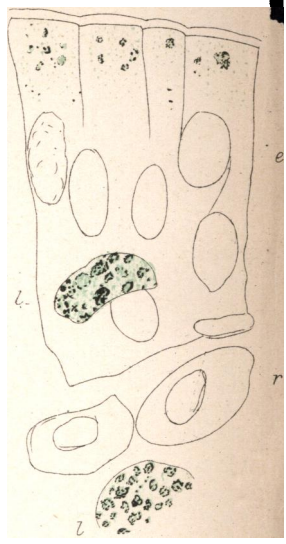

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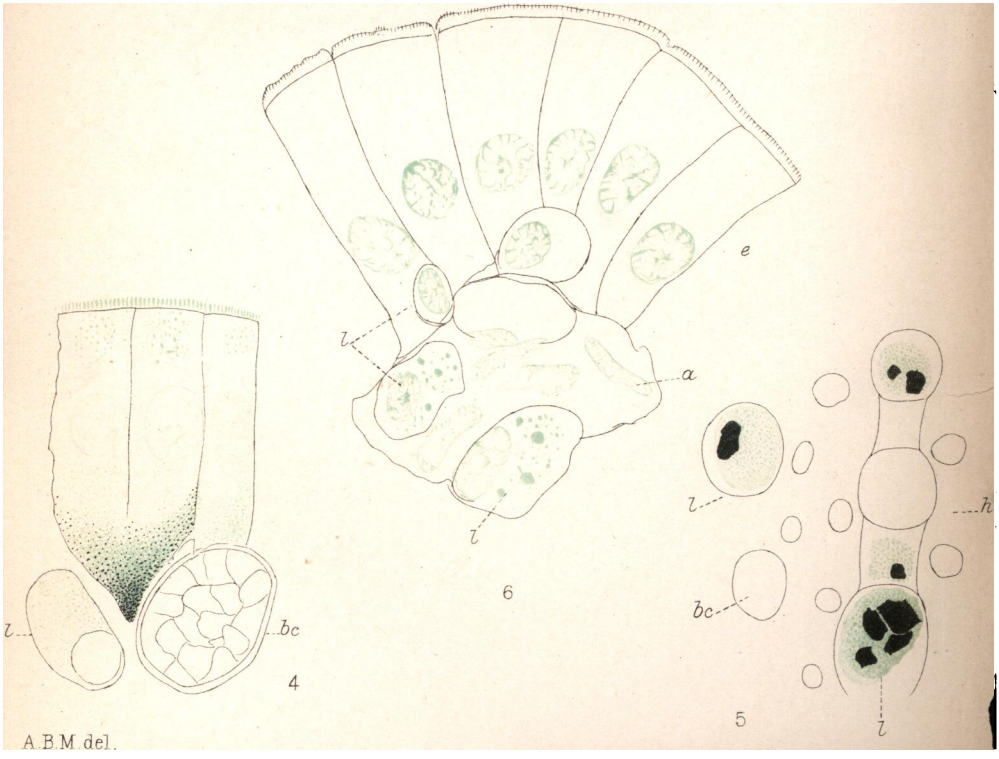

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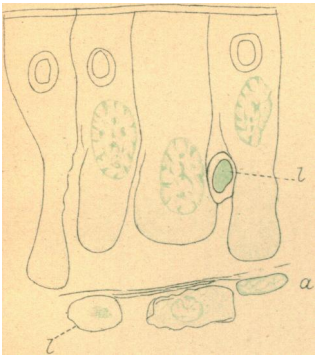

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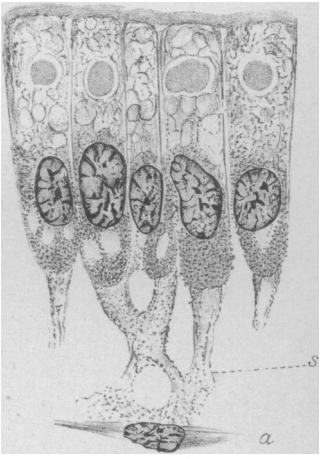

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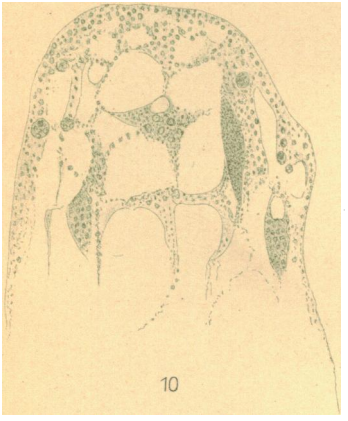

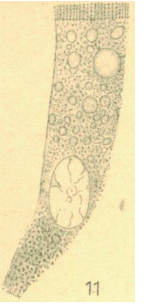

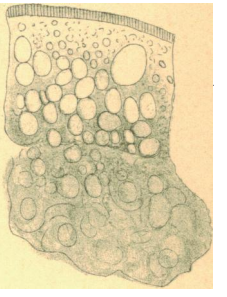

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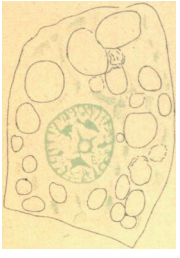

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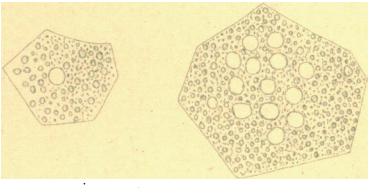

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