There is another point with regard to the relation of the peritoneum to implantation and hematogenous infections. A few years ago I observed a rather extraordinary specimen of carcinomatosis in which there had been widespread hematogenous dissemination of the growth. In that case there were pearly masses of neoplastic tissue in the lung and on the surface of the pleura; one could see very readily that even the small groups of cells quickly passed through the thin serosa; so that while the passing of the peritoneum over the growth would be quite conclusive evidence that infection was lymphogenous or hematogenous, the absence of serosa over the nodule would not be proof of implantation. Within the last two years I exhibited before the Philadelphia Pathological Society an extraordinary instance of metastasis of mammary carcinoma. In that case many of the masses would be found with branching fragments of the serosa running directly into the nodule. There were many of them small, not larger than a pinhead and even not elevated. A microscopic examination would show that the serosa entered the periphery of the nodule in a fragmentary way. In the extension of both carcinoma and sarcoma from one serosa to another, from the peritoneum to the pleura, or the reverse, or to the pericardium, or the reverse—the plugs of neoplastic cells may pass directly through the communicating lymph channels which recently have been studied most carefully by Küttner; in such cases the extension is directly through, the neoplasm appearing on the surface of the two serosæ like a collar button, the shank of which corresponds to the intradiaphragmatic part of the growth and on either side is the new tissue covered by the serosa.

THE CHARACTER OF CHROMATOPHORES.*

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In regard to the origin of the cell masses characteristic of pigmented moles and in regard to the origin of the melanotic tumors of the skin, different views are still held. A number of pathologists believe in the epithelial origin of nevi and of melanotic tumors; others assume a mesodermal origin. The latter view is especially represented in the work of Ribbert.¹ He believes the melanotic tumors to be composed of in part modified chromatophores. All chromatophores he regards as of common mesoblastic origin, in accordance with the previously formulated theory of Ehrmann.² In view of this divergence of opinion, it might not be without interest to inquire what the actual stand of our present knowledge of the chromatophores is, and if it supports the view of Ehrmann and Ribbert. Without attempting to give a complete review of the literature on this subject, I shall limit myself to a brief résumé of the more important results obtained and of some of the problems still to be solved, mainly considering here the chromatophores found in the skin of vertebrates.

They may be found in the epidermis, as well as in the cutis of different classes of animals. The situation, size, number and ramification of processes of the chromatophores vary in different animals. In the cutis of the frog, e. g., there is usually a well-marked layer of chromatophores, which are generally much larger and frequently more branched than the epidermal chromatophores, whereas the guinea-pig cutis has no well-developed cells of this kind, and its pigment is distributed irregularly in masses or clumps; sometimes only

small granules are found. The dermal chromatophores of the frog are separated from the epidermis by considerable connective tissue and the epidermal chromatophores are usually situated higher up in the epidermis than is the case in the guinea-pig. Different classes of animals and different structures show typical differences in regard to their chromatophores.

Let us consider if chromatophores, which frequently have a relatively small cell body and an extremely large number of irregularly drawn out ramifications, are cell entities or not. This has been denied by Schwalbe, who believed that the branched processes of chromatophores are nothing but the interstices between the epithelial cells, filled with pigment. It was also denied by Kromayer, who interpreted the ramifications of chromatophores as parts of protoplasm, belonging to a number of epithelial cells. The epithelial fibrillæ may extend continuously through several cells. This may be especially marked during certain stages of the regeneration of the skin. Kromayer believed the pigment to be derived from such fibrillæ. That chromatophores with their ramifications correspond to individual cells becomes, however, clear, if we study the regeneration of the pigmented guinea-pig skin. There the chromatophores appear first as a kind of ameboid cells, not yet very much pigmented. Under these conditions it can be seen that the processes are extensions of the protoplasm of one epithelial cell.

If, then, the chromatophores are cells, what kind of cells are they? Let us consider the chromatophores of the epidermis. The former view held by Aebi and others that they are ordinary leucocytes or connective tissue cells which migrated into the epidermis has been entirely abandoned. At present it is a question whether they are of epithelial origin or a specific variety of mesodermal cells growing from the cutis into the epidermis, or distributing their pigment from the cutis into the epidermis. The origin of chromatophores in developing skin has among others been traced in the larvæ of the frog by Kodis,³ and in the developing feather by Post⁴ and Strong.⁵ They found epithelial cells gradually taking on the character of chromatophores. In mammals, only recently Maurer⁶ described the first appearance of pigment in the skin in typical epidermal cells. Indeed, if we examine specimens of a developing feather, there can be no doubt that the chromatophores are transformed epithelial cells. In a similar way the chromatophores of the regenerating skin of the guinea-pig have at first a distinctly epithelial character. We see here clearly that the epidermal chromatophores regenerate in conjunction with the other epithelial cells. We find mitoses in chromatophores mainly at such places where other nearby epithelial cells divide mitotically. The same stimulus affects both kinds of cells in the same manner. The regenerating epidermis carries at a certain stage its pigment almost exclusively in chromatophores. Gradually the other epithelial cells become pigmented; the cutis beneath the regenerating epithelium receives its pigment later than the regenerating epidermis; an ingrowth of mesodermal cells into the epidermis can with certainty be excluded.

Similar conditions are found in the regenerating skin

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1. Ribbert: *Lehrbuch der allgemeinen Pathologie*, Leipzig, 1901.

2. Ehrmann: *Bibliotheca Medica Kassel*, 1896.

3. Kodis: *Epith. u. Wanderzelle in der Haut des Frowhlarvenschwanzes*, Archiv f. Anatomie u. Physiol., 1889.

4. Post: *Ueber normale u. pathol. Pigmentierung der oberhautgebilde*, Virchow's Archiv, vol. cxxxv.

5. R. M. Strong: *The Development of Color in the Definite Feather*, Bull. Mus. Comp. Zool., vol. xl, 1902.

6. Maurer: *Die Epidermis und ihre Abkömmlinge*, Leipzig, 1895, and *Festschrift f. Haeckel*, Jena, 1904.

of frogs. The chromatophores in the regenerating skin are epithelial cells, which regenerate simultaneously with other epithelial cells. They divide mitotically, where and when ordinary epithelial cells divide by mitosis. In the first two weeks of regeneration they frequently lose their processes and have thus the appearance of highly pigmented epithelial cells which are often pushed into the upper rows of the thickened regenerating epidermis, and are then cast off with the other cells. In this case, too, the corium beneath the regenerating epithelium is for a long while entirely devoid of pigment cells and an immigration of dermal cells into the epidermis can be excluded. During mitosis the arrangement of the pigment in guinea-pigs and frogs is similar to the distribution of the pigment in ordinary pigmented epithelial cells; we find it in a concentric layer around the chromosomes. Chromosomes and pigment are separated by a light zone. The chromatophores, however, contain more pigment than the ordinary dividing epithelial cell. The processes may be present or may have disappeared during mitosis. In one respect, however, the regenerating skin of the frog differs markedly from the regenerating guinea-pig skin. The latter shows a great regularity in the casting off of the old pigment and in the formation of new pigment. Distinct periods can be distinguished during this process. The skin of the frog is entirely devoid of such a regularity.

A question of some interest is, if during regenerative processes in the adult skin, chromatophores are newly formed from ordinary epidermal cells. I am inclined to believe it, because in transplanted black skin of the guinea-pig there exists a period when almost all pigment has disappeared and only a number of chromatophores can be found which are slightly pigmented and have relatively small processes. It is not unlikely that they represent ordinary epithelial cells in the process of transformation into chromatophores, though it is difficult to give the actual proof of such a new formation.

If, then, the epidermal chromatophores are of epithelial origin, what is the origin of the dermal chromatophores? It is usually assumed that they are modified connective tissue cells. Kromayer believes that they are also derived from the epithelium. Such an origin has lately been made very probable in the case of the chromatophores of the choroid which Lewis found to be derived from the pigmented epithelial cells of the optic cup.

In the regenerating frog-skin I observed repeatedly that the epithelial chromatophores may be pushed into the underlying coagulum, under conditions in which the reverse process, namely, an ascending movement of dermal chromatophores into the epidermis, could be with certainty excluded. The possibility of an epithelial origin of the dermal chromatophores is also suggested through Maurer's⁶ investigations in the formation of the cutis in amphibia. The question, however, can not as yet be regarded as decided. Whatever origin the dermal chromatophores may have, it is certain that during regeneration of the frog-skin they behave very differently from the epidermal chromatophores. The latter cover the wound very soon, moving with the regenerating epithelium, while the former seem to be extremely inert; chromatophores do not appear in the cutis until after two or three weeks, though regeneration begins here at the end of five days. In fact, the subepidermal part of the wound is filled with connective tissue before any chromatophores are to be seen in it. The chromato-

phores of the dermis were not yet regularly arranged at the end of thirty-four days. They were missing at some places and at other points they were situated deeper than is the case normally. They appear occasionally in increased numbers at the margin of the wound. In a similar way the pigment of the cutis of the regenerating skin of the guinea-pig appears later than that of the epidermis, as was mentioned above.

If the epidermal chromatophores originate in the epidermis, it might nevertheless be possible that the epidermal pigment is carried into the epidermis through the chromatophores of the cutis. This, however, can be excluded, because in various classes of vertebrates the first pigment was found in the epithelium, and not in the cutis.

One more question remains to be considered. What are the relations of the epidermal chromatophores to the other epithelial cells? Pigment can originate in ordinary epithelial cells. The retinal pigment is an instance. According to Schwalbe, during the change of hair of *Putorius erminea* chromatophores can not be found in any epithelial or connective tissue structure. In this case the pigment is found in ordinary epithelial cells. On the other hand, there exists a great probability that in some other cases the epidermal chromatophores do actually transfer the pigment granules or rods to other epithelial cells. This seems to take place in the feathers of some birds. There the pigment is at first only found in chromatophores which send out long processes. These processes come in contact with epithelial cells which may be situated very far from the cell-body of the chromatophore. These cells become now gradually pigmented, the pigment being located around the cell nucleus on the side toward the process of the chromatophore. In the skin of the frog or of the guinea-pig it is not possible to see similar clear connections between the function of the chromatophores and the pigmentation of ordinary epithelial cells. During regeneration and transplantation of the pigmented skin of the guinea-pig there is a period when the old pigment and most of the old epithelial cells have been cast off. Simultaneously with the new basal pigment there appear only slightly pigmented chromatophores. It is very difficult to decide if the first basal pigment appears only in chromatophores or also in ordinary basal epithelial cells. Very soon, however, the epithelial cells contain pigment also at a time when the chromatophores are yet very little pigmented. Under these conditions it appears not likely that the chromatophores provide the pigment of the other epithelial cells. Furthermore, the basal epithelial cells contain pigment around the whole periphery of the nucleus; the pigment of all other epithelial cells is found only at the outer side of the nucleus toward the horny layer. The pigment is arranged in a similar way in the cells of the feathers, where, in all probability, the pigment is carried into the cells through the chromatophores. The fact that the basal cells show a different distribution of the pigment seems to me to point to the conclusion that the pigment originates here, just as well as in the chromatophores, which have also a basal situation. This view seems to be supported by the fact that pigment is located around the nucleus. From Arnold's investigations it appears as though just at this place synthetic processes, as, e. g., the formation of fat, would take place. At least here are the first fat droplets to be found. Lillie showed that at the same place oxydative processes do probably take place. As we shall see presently, it is likely that oxydative processes play an important part in the formation of the pigment of the skin. It

might, therefore, be suggested that here pigment is formed through the metabolic processes of the cell, if it were not for the fact mentioned above, that a similar position of the pigment at one side of the nucleus is to be found in cells which very probably do not produce the pigment, but receive it from other sources. How these two apparently opposing facts can be reconciled will have to be determined by further investigations. We may, however, state that, with great probability, the relations of the chromatophores to the ordinary epithelial cells is somewhat different in different classes of animals.

The pigment originates in the epidermis, and the production of melanin is a peculiarity of certain epithelial cells which preserve this function if they are transplanted to a place where formerly non-pigmented cells were present. The melanin in the chromatophores is probably derived from the proteid substances of the cell and is very likely not directly produced from a derivative of the hemoglobin for the following reasons:

1. The sulphur content of the melanin is very large.⁷
2. From the melanin decomposition products can be obtained similar to the ones obtained from the melanoidins, which themselves can be produced with the aid of acids from proteid substances.
3. Tyrosin, a radical, present in cell proteids, can, with the aid of an oxydative ferment tyrosinase, be transformed into substances similar to the melanin of sepia, and similar, probably, to the melanins of vertebrates. It is, therefore, not unlikely that the production of the pigment of the chromatophores is due to a fermentation causing oxydative and condensation processes in certain decomposition products of cell proteids.⁸

That the presence of chromatophores and of pigment in the cells is not without consequences is shown by the difference in the regenerative power of the white and pigmented skin of the guinea-pig after transplantation. Whether this difference is due to the different degree of absorption of light, and perhaps of other rays, is yet to be investigated. The theory that all chromatophores are derived from one specialized kind of mesodermal cell, the melanoblasts, I believe I have shown to be without foundation.⁹

DIABETES MELLITUS.

REPORT ON A CASE, INCLUDING A NEW METHOD OF PROGNOSIS.*

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A diabetic patient with a low acidosis and with no trace of albumin in the urine, when put on a meat fat diet, showed a constant ratio of dextrose to nitrogen in

the urine. The ratio was Dextrose: Nitrogen=3.65:1, and is the same as that found in dogs made diabetic with phlorhizin. This ratio was uninfluenced by fat digestion or by fat metabolism. The sugar was, therefore, derived from proteid alone, and rose and fell with the proteid metabolism as indicated by the amount of urinary nitrogen. The patient died five weeks later.

There was apparently a complete intolerance for carbohydrates; 85 per cent. of starch and 80 per cent. of levulose fed were excreted as sugar in the urine. Withdrawal of the carbohydrates from the diet and the continuance of the same proteid-fat quantity in the food had no effect on the excretion of nitrogen in the urine, which indicates a complete intolerance for carbohydrates.

A sufficient quantity of meat and fat for the body's needs was readily assimilated. Only 4 per cent. of the available energy of the food appeared in the feces.

The urea elimination was normal. The sulphate excretion ran normally proportional to the proteid metabolism. The phosphates ran abnormally high, as is usual in diabetes.

The above research, when considered in the light of other work to be found in the literature, enables us to formulate a new clinical method of prognosis in diabetes, using the D:N ratio for this purpose.

The procedure is as follows: If a diabetic be put on a meat-fat diet (rich cream, meat, butter and eggs), and the twenty-four-hour urine of the second day be properly collected,¹ the discovery of 3.65 grams of dextrose to 1 gram of nitrogen signifies a complete intolerance for carbohydrates, and probably a quickly fatal outcome. We have called this the fatal ratio. A lower relation between dextrose and nitrogen means that some proteid sugar may be burned. Such a tolerance for sugar may be increased on a meat-fat diet, so that the D:N ratio falls, or the sugar in favorable cases may entirely disappear from the urine.

DISCUSSION.

DR. VICTOR C. VAUGHAN, Ann Arbor—This paper shows: 1, that proteid overfeeding may easily be done in diabetes; 2, that the dietary as given by von Noorden is not the proper dieting; 3, that one must estimate the total nitrogen; 4, urea determinations are not sufficient; 5, he finds here that his relation between "D" and "N" remains constant simply because they both come from the same thing. They both come from the proteid of the food, and, consequently, where one increases the other increases; when one decreases the other decreases, and the relation remains the same. Some years ago I published some dietary tables for diabetes and I have followed them ever since. The plan consists in cutting down the carbohydrates gradually, generally taking bread only, because we can estimate the amount of carbohydrate in bread easily, taking say 600 grams during the first day and cutting it down a hundred grams a day (supposing that there is about 55 per cent. of carbohydrates in wheat bread), and making good the calories not with nitrogen but with fats, and (some of you will object to this, I dare say) with alcohol. I do not believe in the general use of alcohol in medicine, but I can not treat this kind of diabetes well without alcohol. Alcohol furnishes a large number of calories and one gets no sugar. Dr. Lusk has shown that there is no reason, at least at present, for believing that any of the dextrose comes from the fats. I think we have been influenced altogether too largely in this country by von Noorden's dictum on diabetes, and I think it has done us harm.

1. The urine should be collected so that an early morning hour (before breakfast) terminates the period for one day. This is necessary, because the sugar formed from eaten proteid is eliminated before the nitrogen belonging to the same. The long period between the evening meal and breakfast allows for the elimination of both constituents.

7. Abeland Davis: Journal of Exper. Medicine, vol. i, 1896.

8. Of interest in this connection is the observation of Jacques Loeb, that the disappearance of black pigment of the yolk sac of fundulus embryos can be demonstrated if there is lack of oxygen.

9. Other references to be consulted are: L. Loeb: Untersuchungen über die physiolog. Wirkungen des Sauerstoffmangels, Pflüger's Archiv, vol. lxi. L. Loeb: Ueber Transplantation von weisser Haut auf einen Defect in schwarzer Haut u. umgekehrt am Ohr des Meerschweinchens, Arch. f. Entwicklungsmech, vol. vi, 1897. L. Loeb: The Growth of Epithelium in Agar and Blood Serum in the Living Body, Journal of Medical Research, vol. viii, 1892. L. Loeb and R. M. Strong: Regeneration of Pigmented Skin of the Frog, Amer. Jour. of Anatomy, vol. iii, 1904.

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