places, and it seems clear that, if his carmine gelatin could penetrate through these, so could an equally viscid substance, and with still greater ease, the blood-plasma. As "no injection in the intercellular bile-canaliculi nor in the perivascular lymphatics nor between the cells" could be detected, the penetration of the gelatin can hardly be ascribed to undue stress. "There being," also, "no diffusion of carmine nor any staining of the cells or nuclei by carmine," the nucleo-mural net-work to which I refer must be an independent structure, circumscribing two kinds of cavities: the canals and the vacuoles. The canals communicating with the exterior of the cell, they are probably the receiving cavities, while the vacuoles, their neighbors, are the spaces in which the *useful* products of metabolism are accumulated. The canals themselves, continuing until Kupffer's vesicle is reached, would thus pour their excretory contents—bile and its various constituents—into this cavity, and this, in turn, would convey them to the intercellular bile-capillaries through its own canals.

Whether so direct a connection between the intercellular capillaries and the bile-channels through the cell exists is a point to be determined. Bile and the various bodies excreted with it would be voided as are the intestinal contents, the canalicular walls taking up certain elements, while physiological substances would be mixed with the substances in transit for definite purposes.

The first question that suggests itself is the following: Is glycogen formed during the *active* functional activity of the liver (during digestion) or during its *passive* state (between meals)? We have seen that the production of urea is increased immediately after a meal; we have evidence, therefore, that an active state based upon increased oxidation processes must prevail, and that it is during digestion that the substances out of which glycogen is formed reach the liver, *i.e.*, while the oxidizing substance is present in the capillaries. This suggests that the oxidizing substance must itself take part in the formation of glycogen, though perhaps *indirectly*, and also in the elaboration of bile.

The main coloring constituent of bile, bilirubin, I have previously considered as the product of a reaction in the intercellular capillaries. As such it is probably eliminated with the bile merely because its high oxygen constituent places it beyond the limits of further oxidation. Yet we have in another component of bile, biliverdin, evidence that some oxidizing process occurs during the passage of bilirubin through the cell, under certain circumstances,—perhaps when more oxidizing substance is present,—for Howell says: "Biliverdin is supposed to stand to bilirubin in the relation of an oxidation product. Bilirubin is given the formula, $C_{16}H_{18}N_2O_3$, and biliverdin, $C_{16}H_{18}N_2O_4$, the latter being prepared readily from pure specimens of the former by oxidation."

With this evidence that oxidation does play some rôle of the processes involved, it will facilitate my task to briefly review the mutual relations of main biliary constituents. By far the larger proportion of these is made up of the bile acids, namely: the sodium salts of cholic acid,—*i.e.*, glycocholic and taurocholic acid. These are obtained from cholic acid *derived itself from sugars and fats* (Voit). Now, Tappeiner[19] found that cholic acid yielded fatty acids *on oxidation*, and, since taurocholic and glycocholic acids are fatty acids, this suggests that they become such during their passage through the hepatic cells and as the result of an oxidation process.

The various phases of the process in the hepatic cells become clear when the oxidizing substance is included as one of the intrinsic factors involved. The blood-plasma of the portal vein contributes the sugars and fats (along with the waste-products to be excreted), while the hepatic artery supplies plasma containing the oxidizing substance. All three active agents meeting in the canaliculi, a part of the sugar (according to the proportion of oxidizing substance present) and all the fat (if the proportion of oxidizing substance is not abnormally reduced by suprarenal insufficiency) are oxidized into cholic acid. But, as the blood also contains glycocoll (probably collagen-cartilage, mucin, connective tissue, and gelatin-waste), glycocholic acid is formed. Again, since the blood likewise contains taurin (probably muscle and pulmonary-tissue waste), taurocholic acid is formed. Just the amount of oxidizing substance necessary being supplied by the hepatic

[19] Tappeiner: Zeitschrift für Biologie, Bd. xii, S. 60, 1876.

artery to each lobule, to properly regulate the functions involved, only the required sugar is burnt by the oxidizing substance. The rest, under the influence of the nuclei of the hepatic cells and the mural protoplasm of the latter, is converted into glycogen and collected in the adjoining alveoli.

But we must also account for the elimination of the many waste-products that are found in bile. An interesting feature connected with these fatty acids is that they can combine synthetically with other bodies, even with proteids, while they are simultaneously able to emulsify the more insoluble soaps and other fatty acids and thus insure their elimination. Again, cholesterin, mainly derived from the white matter of the cerebro-spinal axis and nerves (Flint), in which it occurs in abundance (Foster), was formerly considered as a fatty substance capable of undergoing saponification, but it is now classed among alcohols: the only alcohol that occurs in the organism in a free state. This body is not only soluble in solutions of the biliary acids also, but it *combines* with acids, including glycocholic acid. The importance of this fact appears when it is recalled that insufficiency of glycocholic acid in this connection—and also, perhaps, of oxidizing substance—is the main source of gall-stones. The cholesterin being a constant constituent of bile, when there is not enough glycocholic acid present to take it up, it is precipitated in the gall-bladder and there forms the calculi of which it is the main component. Another body derived from nervous structures, but which, like cholesterin, is to be found in other fluids, especially blood-serum, is lecithin. This body, besides others not mentioned, only occurs, however, in very limited proportions.

It is now evident that glycocholic acid and taurocholic acid should be looked upon as *functional* acids, in the sense that they are not only vehicles for waste-products of metabolism, but are also capable of submitting them to dissociating reactions under the influence of the oxidizing substance. They are sources of energy precisely as myosinogen appears to be a source of energy, and capable of becoming factors of combustion phenomena when in contact with the latter substance. They are also truly physiological in the sense that they serve to recover or economize those products which can again be

used by the organism. Indeed, they (or at least a part of their decomposition products) are again absorbed by the intestinal mucous membrane, and, passing through the venous channels, probably take up therein and transfer to the hepatic cells what waste-matter they are to carry to the intestinal tract. We thus have in the cellular canaliculi two acids endowed with powerful affinities and an active reagent, the oxidizing substance, to account for the processes of a chemical nature connected with the functions of the hepatic cell.

Of course, all this involves the necessity of showing, as a controlling factor, that products of combustion are also present. The following lines by Professor Howell give this feature due emphasis; referring to bile, he says: "The secretion contains also a *considerable, though variable, quantity of* CO_2 *gas,* held in such loose combination that it can be extracted with a gas-pump without the addition of acid. The presence of this constituent serves as an indication of the extensive metabolic changes occurring in the liver-cells."

Again, the element of nervous control implied when I referred to the oxidizing substance contributed by the hepatic artery must be shown. We have seen that the vagus was the *active* nerve during functional activity of the stomach; that it should likewise govern hepatic functions is obvious. That such is the case is sustained by no less an authority than Claude Bernard, to whom we owe the discovery of glycogen—one of his greatest achievements—and whose conceptions have been almost all sustained by all the labor bestowed upon them since. He not only found that the vagus was the predominating nerve in the liver, but that its section also suppressed its glycogenic function.

The fate of glycogen, its conversion into sugar for the use of muscular and other tissues, may now be analyzed with greater facility.

My analysis of muscular functions led me to the deduction that the contractile elements contained a substance, myosinogen, which, when brought into contact with the oxidizing substance of the plasma, became the source of the muscle's working energy. Ample evidence was afforded to show that we were dealing with an oxidation process, the intensity of which

was commensurate with the amount of blood that the arterioles supplied to the contractile tubular elements. There seems to be considerable analogy between this process and that which prevails in the hepatic lobule. Howell alludes to this in the following words, which well recall the fact that we referred to glycogen as the main constituent of myosinogen: "The history of glycogen is not complete without some reference to its occurrence in the muscles. Glycogen is, in fact, found in various places in the body, and is widely distributed throughout the animal kingdom. It occurs, for example, in leucocytes, in the placenta, in the rapidly-growing tissues of the embryo, and in considerable abundance in the oyster and other mollusks. But in our bodies and in those of the mammals generally the most significant occurrence of glycogen, outside the liver, is in the voluntary muscles, of which glycogen forms a normal constituent."

The similarity between muscular and hepatic sources of energy is further emphasized when, in the following paragraph, Howell says: "In accordance with the view given above of the general value of glycogen—namely: that it is a temporary reserve-supply of carbohydrate material that may be *rapidly converted into sugar and oxidized,*[20] *with the liberation of energy*—it is found that the supply of glycogen is greatly affected by conditions calling for increased metabolism in the body. Muscular exercise will quickly exhaust the supply of muscle and liver glycogen provided it is not renewed by new food. In a starving animal glycogen will finally disappear, except perhaps in traces; but this disappearance will occur much sooner if the animal is made to use its muscles at the same time. It has been shown also by Morat and Dufourt that, if a muscle has been made to contract vigorously, it will take up much more sugar from an artificial supply of blood sent through it than a similar muscle which has been resting; on the other hand, it has been found that, if the nerve of one leg is cut so as to paralyze the muscles of that side of the body, the amount of glycogen will increase rapidly in these muscles as compared

[20] The italics are my own.

with those of the other leg, that have been contracting meantime and using up their glycogen."

These facts clearly indicate that oxidation processes are not in order here, since glycogen is a source of energy, intended, therefore, for subsequent oxidation wherever it is distributed. Indeed, Professor Foster remarks, in this connection: "It was formerly believed that this sugar underwent an immediate and direct oxidation as it was circulating in the blood. . . . It is sufficient for us at the present to admit that the sugar is made use of in some way or other." Referring to the physiological uses of glycogen, he also says: "The main purpose of the deposition of glycogen is to afford a store, either general or local, of carbohydrate material, which can be packed away without much trouble so long as it remains glycogen, but which can be drawn upon as a source of soluble circulating sugar whenever the needs of this or that tissue demand it." That the oxidizing substance has nothing to do with the process is clear.

The conversion of glycogen into sugar in the liver appears as a wasted function, the carbohydrates having been already split into dextrose or dextrose and levulose in the portal system. Why they do not merely pass on to the several tissues seems strange. But it soon becomes evident that, were it otherwise, sugar would accumulate, then be excreted by the kidneys, and lost, since there can only be a fixed and limited (0.1 or 0.2 per cent.) amount of sugar in the circulation at a given time. So useful a substance is, therefore, stored, after dehydration, in the hepatic cell as glycogen, and converted into sugar according to the needs of the organism.

Conversion of the liver glycogen into dextrose is generally ascribed to a special ferment, thought to originate in the liver, but the nature of which has remained undetermined. The experiment of Claude Bernard, upon which this view is mainly based, is the following, as related by Stewart: "A rabbit, after a large carbohydrate meal—of carrots, for instance—is killed and its liver rapidly excised, cut into small pieces, and thrown into acidulated boiling water. After being boiled for a few minutes the pieces of liver are rubbed up in a mortar and again boiled in the same water. The opalescent aqueous extract is

filtered off from the coagulated proteids. No sugar, or only traces of it, is found in the extract, but another carbohydrate —glycogen, an isomer of starch, giving a port-wine color with iodine and capable of ready conversion into sugar by amylolytic ferments—is present in large amount. Again, the liver, after the death of the animal, is left for a time *in situ*, or, if excised, is kept at a temperature of 30° to 40° C. or for a longer period at a lower temperature; it is then treated exactly as before, but no glycogen, or comparatively little, can now be obtained from it, although sugar (dextrose) is abundant. The inference plainly is that after death the hepatic glycogen is converted into dextrose by some influence which is restrained or destroyed by boiling. This influence may be due to *an unformed ferment* or to the *direct action of the liver-cells*, for both unformed ferments and living tissue-elements are destroyed at the temperature of boiling water."

Another explanation suggests itself to me if, instead of a ferment of hepatic origin, we hypothetically use one of external origin: In the first procedure immediate immersion in boiling water destroyed the ferment which happened to be in the blood-vessels, while, in the second, the ferment was given time to act. The difference in the conclusions vouchsafed is simply this: no thought being given to the blood-vessels, the ferment could only be considered as of cellular origin. We have seen how many functions ascribed to the hepatic cells really belonged to the intercellular blood-stream; this seems to be an additional one.

Admitting that we are dealing with a ferment of external origin, from which organ could we expect it to be derived? Can we attribute the process to ferments from the salivary glands or pancreas? If it is produced only by digestive ferments,—*i.e.*, amylolytic ferments poured out during digestion, —why does glycosuria appear irrespective of any digestive process when the floor of the fourth ventricle is punctured, as shown by Claude Bernard? He also found that conversion of glycogen into sugar was a continuous process, carried on to subserve the needs of the organism: a perfectly logical conclusion if the liver is really a storehouse for this substance. Again, the quantity of sugar in the blood, as we have seen,

is small, but constant. How could we account for these features of the problem with ferments transmitted through the digestive tract?

Finally, sugars thus produced—*i.e.*, from amylolytic ferments secreted by the digestive tract—do not seem to be dextrose, the sugar produced by the supposed hepatic ferment. Thus, Professor Foster says: "In the case of the amylolytic ferment of saliva, pancreatic juice, intestinal juice, and, indeed, of all other amylolytic animal fluids, the sugar into which starch or glycogen is converted is *maltose*. Now, the sugar which appears in the liver after death is dextrose, identical, as far at least as can at present be made out, with ordinary *dextrose*." It is evident that a ferment other than the amylopsin connected with the digestive process must be the active one, and that it must reach the liver by a channel other than the intestinal tract, the villi, etc. Again, it must be very nearly similar to the salivary and pancreatic amylolytic ferments. The salivary glands are so remote anatomically that they can hardly be considered; we are brought, therefore, to the pancreas as the only organ which could act as source of a ferment or diastase having for its main function to convert glycogen into dextrin.

As shown by von Mehring, Minkowski, and de Dominicis, removal of the pancreas causes marked glycosuria, and this persists whether the animal be given carbohydrates or not. All the other symptoms of diabetes mellitus appear,—namely: increased flow of urine, considerable urea, acetone, etc.; great thirst and hunger, emaciation, marked muscular weakness,— followed by death in two to four weeks. Indeed, we are vividly reminded of the suprarenal glands, on ascertaining that grafting of a piece of pancreas in the abdomen or skin will arrest the glycosuria, and that, if a small portion of the organ is left, the symptoms will disappear. Again, whether carbohydrates are given as food or not, the glycogen disappears from the liver. "We may believe, from these experiments," says Howell, "that the pancreas produces a substance of some kind that is given off to the blood or lymph, and is either necessary for the normal consumption of sugar in the body or else, as is held by some, normally restrains the output of sugar from the

liver and other sugar-producing tissues of the body. What this material is and how it acts has not yet been determined satisfactorily." That we are dealing with an internal secretion is clear, and, such being the case, the secretion probably passes out into the blood by the pancreatic vein, which "opens into the splenic and mesenteric veins." As these open, in turn, in the portal vein, the pancreas would then supply a special ferment for the conversion of glycogen into a functional sugar.

If the foregoing symptoms are closely scrutinized, it soon becomes apparent that the functions of the pancreas—as will be shown in the next chapter—are far more important than is generally believed. For the present, however, we will limit our inquiry to the subject in point.

The fact that, notwithstanding the ingestion of carbohydrates, the glycogen will totally disappear from the liver is easily accounted for. A prominent function of the pancreas during intestinal digestion is to transform starches into maltose, to insure absorption of this sugar. When the organ is removed, therefore, its amylopsin is no longer furnished to the intestinal contents, starches are not properly converted, and the portal vein carries no maltose to the hepatic lobule. The production of glycogen, therefore, ceases. The fact, however, that we can so easily account for this phenomenon suggests that:—

1. The pancreas is the organ upon which all the preliminary functions connected with the formation of glycogen depend.

2. Its amylopsin converts starches in the intestine to prepare them for the elaboration of glycogen in the hepatic cell.

3. Its internal secretion, supplied to the portal system by way of the splenic vein, converts glycogen into dextrose.

That we are on the right path is suggested by a series of experiments by Croftan,[21] in which the conversion of glycogen into sugar was obtained by means of injections of suprarenal extract: "Incomplete as these experiments are," says the author, "they reveal the fact that the injection of suprarenal extract can cause the excretion of dextrose provided the quantity injected *is sufficiently large*. Why in the case of one

[21] Croftan: American Medicine, Jan. 18, 1902.

animal more must be given than in the case of another to produce approximately the same excretion is undecided and remains to be determined." Dwelling upon the presence in the adrenals of a diastatic ferment, he states that "two possibilities may present themselves, viz.: either the suprarenals manufacture a diastatic ferment or they retain the diastatic ferment that is formed elsewhere in the body (pancreas, salivary glands) when it is carried to them in the blood- or lymph- stream." The author also refers to the investigations of F. Blum,[22] who, "testing the effects of suprarenal extract empirically," discovered "glycosuria in 22 out of 25 animals that he operated on." My interpretation of the manner in which these investigators reached their results is, of course, not that of Croftan, since, as I view the process, the oxidizing substance constitutes the active suprarenal agency as a compound of suprarenal secretion and oxygen.

We are dealing with enhanced physiological activity *somewhere*. Indeed, Croftan says: "In order that hyperglycosuria be produced the amount of sugar normally poured into the blood must be increased, or the amount normally destroyed must be decreased." That excessive activity was either procured by the injected extract or by overstimulation of the adrenals, both leading to total insufficiency, is shown by the brief history of one of the animals: "The second rabbit died in one hour and ten minutes; here some *spasmodic* symptoms, involving chiefly the *posterior* extremities, preceded the coma." Referring to two rabbits, including the latter, the first having died in two hours and forty minutes and to "all others to be spoken of presently" (six, all told), he says: "Dextrose was identified in the urine by its cupric-reducing powers and the phenylhydrazin test; in one of the dogs in addition by circumpolarization and yeast fermentation. The substance excreted was undoubtedly dextrose. The amount excreted would be far too large to be explainable by a splitting of the jecorin-like substance mentioned above; it would not, moreover, be possible for considerable quantities of dextrose derived from this source to appear in the urine for several days after the injec-

[22] F. Blum: Deutsche Archiv f. klin. Med., Oct. 31, 1901.

tion." Excessive stimulation by a great increase of oxidizing substance in the blood evidently occurred. As soon as the extract was injected it was carried to the lungs, and lost its individuality immediately therein by taking up oxygen. It could no longer, therefore, act as a diastase.

The question now to decide is this: General stimulation enhanced the production of an amylolytic ferment either by the liver or by the pancreas. To which organ can we ascribe this function? Since oxidation destroys sugar, a great excess of oxidizing substance in the blood would burn sugar actively on all sides and produce the *opposite* of glycosuria: *i.e.*, excessive combustion and rapid disappearance of the liver glycogen through abnormal use of it in the other organs. But here we have, as a result of a great increase of oxidizing substance in the blood, marked glycosuria, and that evidently without preliminary feeding, since this fact is not mentioned by Croftan. As the oxidizing substance does not affect glycogen, that of the liver could not be converted by it into sugar; hence the excessive production of the latter can only be accounted for by an equally excessive production of amylolytic ferment.

Claude Bernard showed that conversion of glycogen into sugar took place more rapidly when the blood was made to traverse the liver with unusual speed. Yet he attributed the formation of the ferment to the liver, having obtained it from pulp rubbed up and treated with glycerin, after the liver had been washed out so as to remove the vascular contents. But it seems clear that injections by the portal vein will hardly deplete the liver of every particle of the ferment in its minute lobular capillaries, while reduction of its substance to pulp and a three days' immersion in glycerin will dissolve all that contained in the latter. When we consider how readily conversion can be produced,—even by traces of soluble albumin, according to Seegen,—it is evident that upon the addition of water to the glycerin solution the very small proportion that may have remained imprisoned in some of the lobules will suffice for the conversion of glycogen.

One of Claude Bernard's experiments seems to me to afford proof that the amylolytic ferment reaches the liver through

the portal vein. By ligating the latter vessel he created a collateral circulation, and shifted the portal-blood stream into the general circulation. Ten or 12 grammes of sugar were then given to the animal, and sugar was soon found in the urine. In a normal dog, on the other hand, 50 to 80 grammes had to be administered before this result was obtained (M. Duval[23]). The absence of sugar in the latter animal's urine until a very large quantity of sugar had been ingested distinctly shows that conversion of its *glycogen* only occurred because its portal vein was open; in the other dog it was not converted glycogen that passed into the urine, but maltose, *i.e.*, intestinal starch which had been submitted to the action of the pancreas's intestinal ferment,—*i.e.*, amylopsin. If we now couple the fact that conversion of liver-glycogen only occurs when the portal vein is free with Claude Bernard's observation that increased speed of the portal blood through the liver causes the glycogen to be converted more rapidly, it seems clear *that the conversion process is not due to an hepatic ferment, and that the pancreas supplies, as an internal secretion, the ferment which converts glycogen into dextrose.*

A perplexing feature of all this requires elucidation, however. If, as we have stated, the blood-plasma contains an oxidizing substance, why is the sugar not oxidized on its way to the tissues of distribution? Armand Gautier[24] refers to the investigations of Jaquet, which demonstrated that sugars mixed with blood containing the oxidation ferment previously referred to, and which we found to be of suprarenal origin, did not become oxidized. He ascertained, however, that upon adding to the blood a small quantity of fine pulp of muscle, lung, or of any other organ, the oxygen was absorbed. This obviously indicates that *dextrose passes through the blood without being destroyed, and it can only become oxidized after combining with bodies produced by the organs to which it is distributed.*

General Functions of the Liver, Spleen, and Pancreas.—All the facts reviewed in this chapter suggest the following conclusions as to the functions of the liver, spleen, and pancreas:—

[23] M. Duval: *Loc. cit.*, p. 378.
[24] Armand Gautier: "La Chimie de la Cellule Vivante," p. 98.

1. The hepatic artery, owing to the oxidizing substance (adrenoxidase) that its plasma contains and the mode of distribution of its terminal capillaries, supplies the exogenous chemical energy which initiates and sustains all reactions in the hepatic lobule that require oxygen.

2. The nervous supply of the liver is composed, first, of terminal subdivisions of the vagus, which enhance the activity of all its functions by causing dilation of the hepatic arterioles; and, second, of terminal subdivisions of the sympathetic, which, by causing constriction of these arterioles, reduce the functional activity of the organ.

3. In the normal subject the liver is anatomically isolated from structures that come into contact with bacteria, and protected against their intrusion by the bactericidal products of the intestinal glands and follicles.

4. The capillaries of the hepatic lobules, owing to the admixture therein of the hepatic artery's oxidizing substance (adrenoxidase) with the portal vein's waste-laden blood, are the seat of several functions now ascribed to the hepatic cell.

5. Blood-pigments and iron, derived from the intestine and spleen, simultaneously penetrate the hepatic lobule, and combine with the adrenoxidase therein to form hæmoglobin. The uncombined pigment is eliminated with the bile as bilirubin.

6. Urea is the end-product of three successive reactions, viz., (1) nitrogenous bodies are reduced to amides in the afferent veins,—mesenteric and portal; (2) the amides are dissociated into ammonia, carbonic acid, and water by the oxidizing substance (adrenoxidase) in the hepatic lobule; (3) urea is formed by synthesis in the efferent veins,—hepatic and vena cava.

7. The hepatic cell contains, besides its vacuoles and nuclei, numerous canaliculi (Schäfer) and a vesicular vacuole which opens into the bile-capillaries by a canaliculus (Kupffer); the canaliculi and the vesicular vacuole are probably connected.

8. Glycocholic acid and taurocholic acid are functional acids, inasmuch as they dissociate and appropriate waste-products, and, under the influence of the oxidizing substance, convert them into excrementitious products in the canaliculi of the hepatic cells.

9. The waste-products so converted by the biliary acids and the latter themselves, constituting bile, are transferred, along with other products for which the latter may serve as vehicle,—bilirubin, earthy salts, etc.,—to the vesicular vacuole of the cell and eliminated by the canaliculus that opens into the bile-capillaries.

10. The biliary acids, blood-pigments, iron, and other bodies or any of their components, that may prove useful to the organism are, entirely or in part, reabsorbed by the intestinal venules and returned to the portal circulation.

11. The sugars converted from intestinal foodstuffs in the intestines are brought to the hepatic lobule with the portal blood, and penetrate the canaliculi with the latter and with the oxidizing substance. During the bile-forming reaction the sugars are dehydrated, and, probably with the assistance of the cellular protoplasm, converted into glycogen.

12. The liver glycogen is converted into dextrose by an amylolytic ferment supplied by the pancreas as an internal secretion, which enters the portal circulation by the splenic vein.

13. Dextrose is distributed to the organs in which it is used as a source of energy by the blood, and only becomes vulnerable to oxidation when combined with products of metabolism furnished by those organs.