

Some problems of intermediary metabolism.

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SOME PROBLEMS OF INTERMEDIARY METABOLISM

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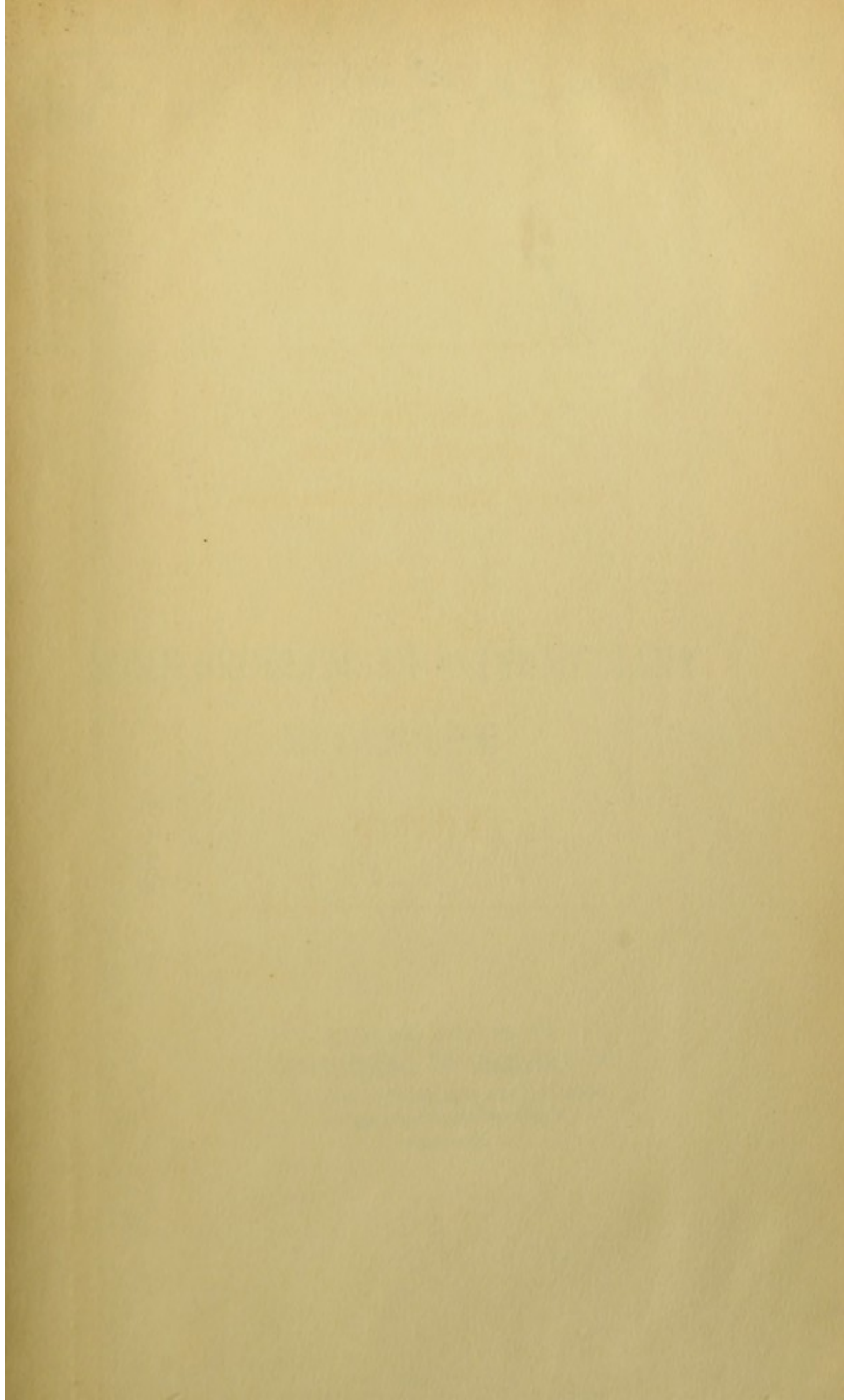
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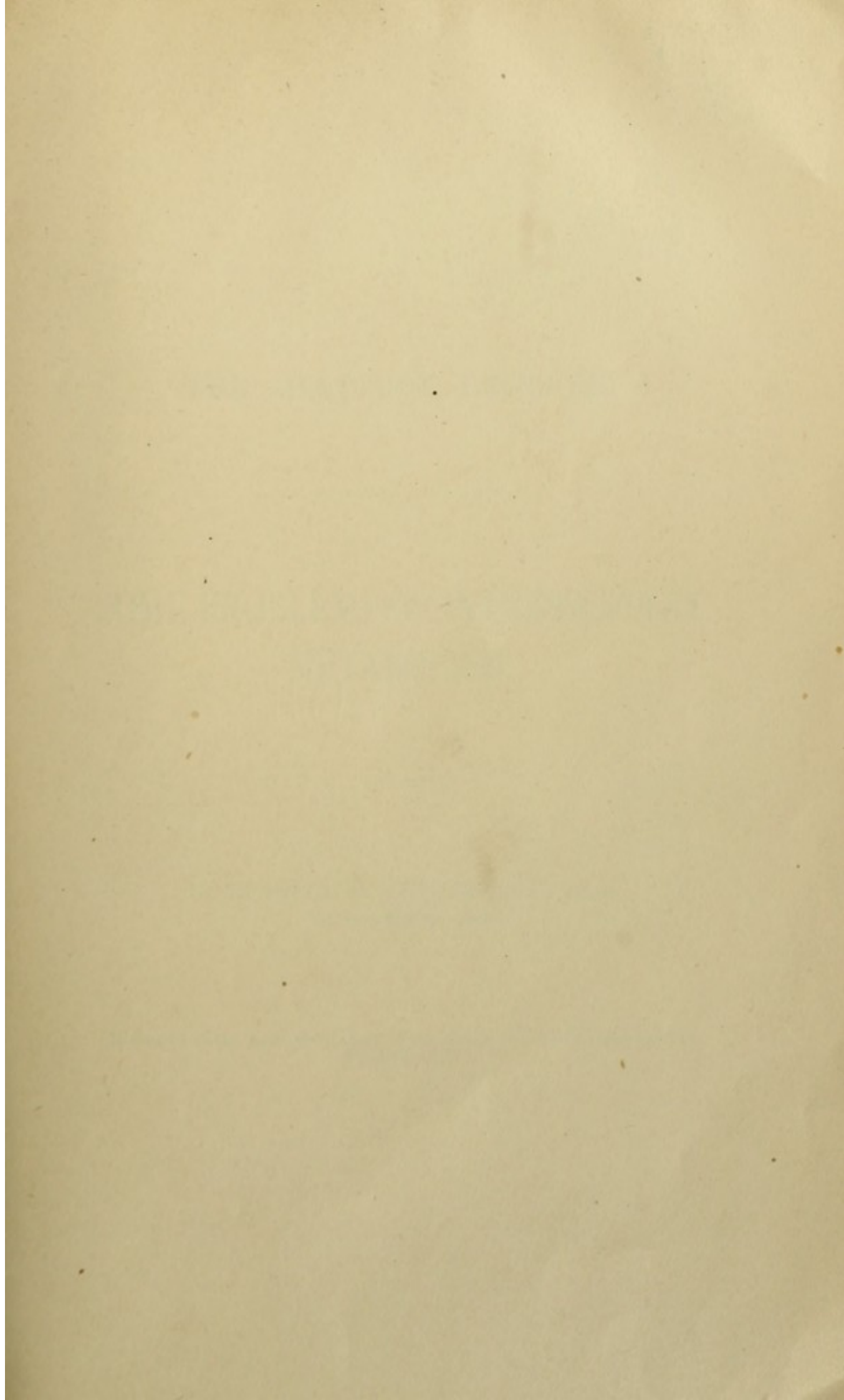
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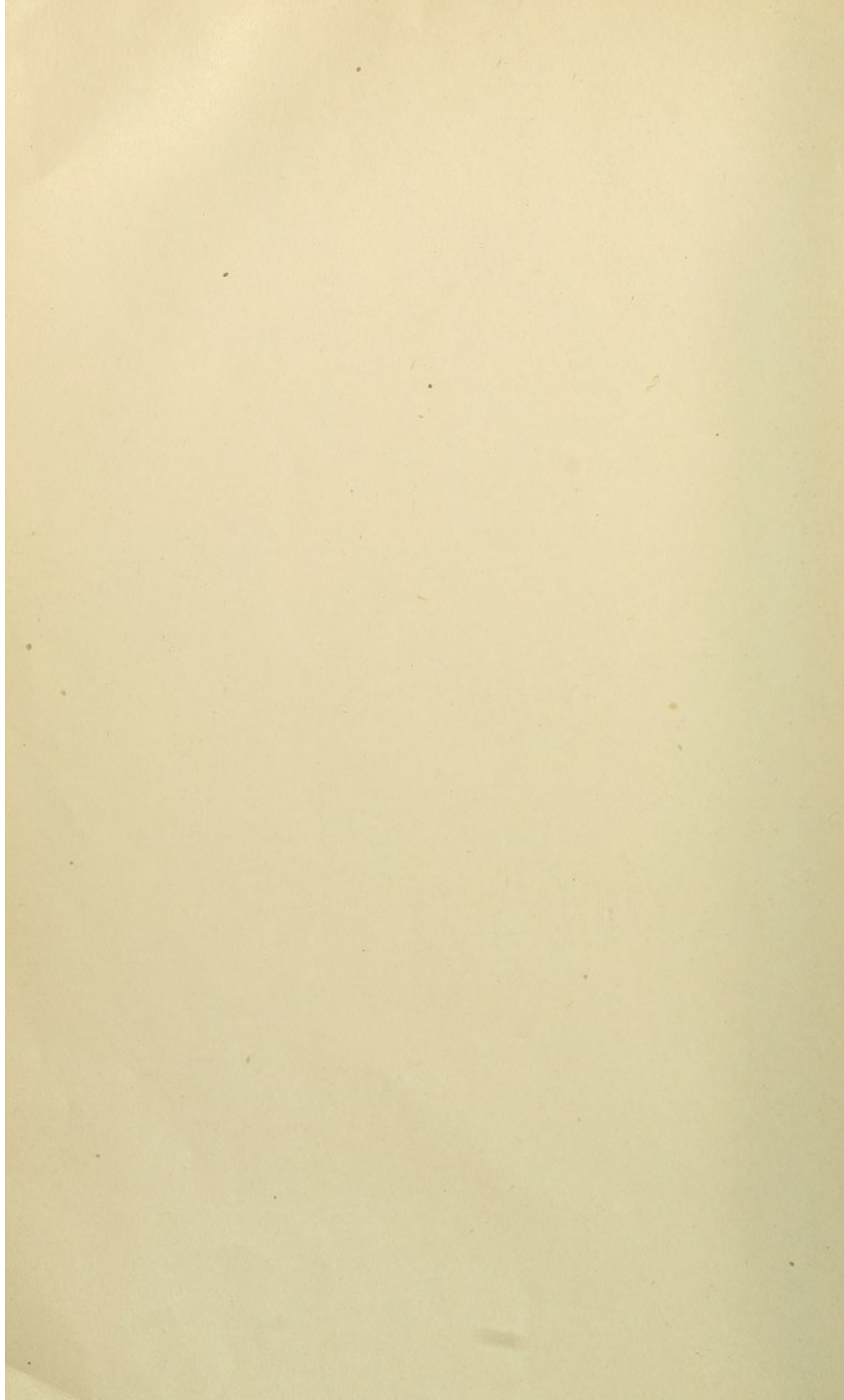
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COLUMBIA UNIVERSITY
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437 WEST FIFTY-NINTH STREET
NEW YORK

Ed
J. H.





THE SHATTUCK LECTURE.

SOME PROBLEMS OF INTERMEDIARY
METABOLISM.

By RUSSELL H. CHITTENDEN, Ph.D.
OF NEW HAVEN, CONN.

Delivered at the Annual Meeting of the Massachusetts Medical Society,
June 13, 1905.

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COLUMBIA UNIVERSITY
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SOME PROBLEMS OF INTERMEDIARY METABOLISM.

It requires little imagination and little knowledge of physiology to comprehend that upon a true understanding and rightful interpretation of the many and varied metabolic phenomena of the body depends in large measure the accuracy of our knowledge of the processes of life, both in health and in disease. Yet as Sir Michael Foster has clearly pointed out, it is wholly impossible at present to make any connected or continuous story of the metabolic changes undergone by the individual constituents of the food, the body or the waste products. In the chain of events from the assimilation of the digested food materials by the hungry tissues down to the elimination of the final waste products by the kidneys, lungs and skin, are many broken and missing links by which the continuity of the story is destroyed and we are compelled to fill the gaps by guesses and conjectures, too often widely divergent from the truth.

The very existence of these gaps and breaks in our knowledge, however, serves as an ever-present stimulus inciting renewed activity in the search for truth and enlightenment. As is often the case, this search brings added complexity by opening up vistas that were not supposed to exist, and our thoughts and our experiments take a new turn, from time to time, as additional facts are brought to the surface, with a suggestion of new methods of interpretation. Advances are constantly being made; old views

are undergoing modifications or are entirely supplanted by new theories, the result of steady progress in knowledge. The gaps and breaks in the chain of events that represents the processes of life are being slowly filled in, but new gaps make their appearance, demanding additional knowledge before we can hope for a perfect understanding of the many and varied steps in the metabolic transformations characteristic of living tissues.

As has been so clearly expressed by many writers, we need full knowledge of *all* the steps in the building up and breaking down of the tissue materials, with full consciousness of the fact that a large majority of the diseases of the body are the result of a perversion of metabolism. Further, we realize full well that it is equally important to possess knowledge of *where* these individual steps in metabolism are located, if we are to have full comprehension of the true functional activity of the individual organs and tissues of the body. We need to know the exact nature of each link in the chain of events, and where each link lies with reference to the other links of the chain. The many degenerative changes that check the activities of man at a time when he should be in the full vigor of life, we frequently say are due to the disorders of metabolism or nutrition, recognizing perhaps full well the general character of the perversion, but with no adequate conception of exactly what has occurred or what should be done to remedy or prevent. We lack knowledge of the intermediary steps in metabolism, in health and in disease, and if we are to interpret aright the conditions that bring about disease, to say nothing of prevention and cure, we must acquire full understanding of the processes of intermediary metabolism, their relationship to each other, their general and specific character and the influences that control their harmonious working.

As has just been stated, many of the gaps and breaks in

our knowledge of metabolism have been filled up, new facts have been brought to light, new suggestions have been made, and our knowledge has been correspondingly increased. It has therefore seemed to me that I could not better fulfil the purpose of the Shattuck lecture than to present to you some of these problems of intermediary metabolism which of late have been under consideration and for which we have now a partial or complete solution.

Every active tissue of the body, every glandular organ, every individual living cell, is the seat of incessant chemical activity; construction and destruction are going on side by side, new food materials — proteids, fats and carbohydrates, together with inorganic salts, water and oxygen — are continually being supplied, and passing into the cells of the various tissues or organs are eventually built up into the tissue material, or are made more or less completely adherent. Simultaneously, the cell substance or the adherent materials are undergoing characteristic changes. Secreting gland cells are manufacturing enzyme antecedents and storing them up in the cells for future use, or are perhaps pouring the products forth in slightly altered form to give character to the glandular secretion. Other gland cells are elaborating specific products, such as bile acids, glycogen, amido-acids of various kinds, internal secretions to be re-absorbed by the blood and serve some purpose elsewhere in the body; decompositions of great variety are being carried on, and complex substances are being broken down into simpler with liberation of energy, manifest in the form of heat or mechanical work. And so we have from every group of individualized cells in the body a more or less continuous stream of katabolic products, representatives of the destructive changes taking place in the cells of the individual tissues or organs. Further complexity is introduced by the fact that a given set of cells may show many different lines of activity. Thus, the hepatic cells

not only manufacture bile acids and bile pigments, but they likewise produce glycogen from sugar and transform sugar into glycogen, they form urea from various amido-acids and ammonia, they bring about the construction and destruction of uric acid, they decompose the hæmoglobin of the blood and withdraw the iron from the hæmatin molecule to manufacture bilirubin, they bring about combinations between sulphuric acid and certain aromatic substances such as phenol; in a word, they are active in carrying forward a great variety of divergent processes in which oxidation, hydration and dehydration are equally conspicuous.

These processes of metabolism may be viewed from the standpoint of ultimate changes, without regard to the few or many intermediary steps in the process. Thus, the physiologist may study, for example, the extent to which the formation of urea goes on in the body under different conditions of diet, etc., and he may acquire much useful information regarding the rate of proteid katabolism, without, however, learning much or anything of the various intermediary steps in the process by which the urea is elaborated from the proteid of the food or of the tissues. It is obvious that we can acquire full and exact knowledge of the way in which urea is formed only by searching out the successive changes that take place from the primary breaking down of the proteid to the ultimate formation of urea; *i. e.*, by studying the intermediary processes of proteid katabolism.

In any consideration of intermediary metabolism, where of necessity the action of specialized groups of cells—as in individual tissues and organs—is involved, we are at once confronted with the general question as to how the processes of destructive decomposition are accomplished. In what manner and by what agencies are the processes of katabolism carried out? Until recent times we have had no logical answer to this question. Physiologists and biolo-

gists long held that the processes going on in living tissues were of far too complicated a nature to admit of explanation by chemical or physical means. Schönbein, for example, maintained that the exact sciences were not sufficiently developed to render possible any exact analysis of the changes taking place in living cells. It was the fashion to assume that it was the functional activity of the living cell as a whole that made possible the chemical changes characteristic of the tissue or gland. Cell activity was considered as the result of an inherent quality of the living protoplasm, and no one agent or definite substance could be held responsible for this activity. The theories of vitalism held the physiologist in a strong grasp. Even when ferments or enzymes gained gradual recognition, their action was considered as purely extra-cellular, and they were not looked upon as playing any special part in metabolism. In digestion, of course, their action was manifest, but it was wholly extra-cellular, though their origin was intra-cellular.

Living cells produced enzymes for certain purposes, but there was no evidence that the ordinary processes of katabolism with which the cells might be involved were connected in any way with ferment or enzyme action. Until very recent times, the yeast cell was taken as a typical example of the then point of view. It produced an inverting ferment which could be extracted from the cell and which was very efficient in inverting cane sugar, but the peculiar property of the yeast cell, *i. e.*, the formation of carbon dioxide and alcohol, was the result of the activity of the cell as a whole. It was considered as due to an inherent quality of the living protoplasm. This view prevailed until a few years ago, when Hans Buchner showed that it was quite possible to press out of the yeast cell a clear fluid which, freed from all cellular elements, would still cause alcoholic fermentation. Still later, an enzyme was isolated—*zymase*—which breaks down sugar into alcohol and

carbon dioxide quite as effectively as the yeast cell itself. In other words, we have learned that this so-called vital property of the yeast cell is due entirely to an intra-cellular ferment or enzyme which the cell manufactures.

In the animal body the processes of metabolism, as judged from the number and variety of products formed, are many and varied. It has been customary to classify these chemical processes under the heads of hydrolytic cleavage, oxidation, reduction, synthesis, etc., and to-day we are in a position to affirm that intra-cellular ferments are the responsible agents in bringing about these varied processes of metabolism in the individual organs and tissues of the body. There is practically no process of metabolism so intricate or obscure that it cannot well be explained by the action and interaction of intra-cellular ferments. New ferments are constantly being discovered, new chemical reactions are being traced to the power of special ferments, and we now speak with great detail of the various *autolytic* changes that different glands and organs undergo, when simply warmed at 40°C. under antiseptic precautions, because of the action of intra-cellular ferments which are practically present in all tissues.

Proteolytic ferments of the trypsin, or of the newly discovered erepsin, type are present in most, if not all, of the tissues of the body. Muscle, liver, kidneys, lymph glands, lungs, spleen, etc., all contain proteid-dissolving ferments, and when the tissues are subjected to auto-digestion or autolysis, such products as the amido-acids, leucin and tyrosin, tryptophan, glycocoll, hexone bases or diamino-acids, and ammonia result from the breaking down of the various proteids of the tissue. In this connection it is to be noted that the respective proteids of the individual tissues are much more quickly affected by these ferments than are foreign proteids, thus suggesting that these intra-cellular enzymes are somewhat different in nature from the ordinary

proteolytic enzymes of the digestive juices. Further, there are peculiar ferments that act specifically upon the intra-cellular nucleoproteids, splitting off the nucleic acid complex from the proteid part of the molecule, and still other ferments that bring about a cleavage of the nucleic acid with liberation of the contained purin bases. Lastly, it may be said that the general trend of action with these intra-cellular proteolytic ferments is hydrolytic cleavage, much the same as the influence exerted by mineral acids or by ordinary digestive enzymes.

Other types of intra-cellular ferments exercising hydrolytic action accompanied by cleavage are to be found in lipases, true adipolytic ferments which split neutral fats into glycerin and fatty acids in a fashion quite analogous to the action of ordinary steapsin. These lipases are found in the liver and other organs. Further, the kidneys and liver contain a ferment capable of splitting hippuric acid into benzoic acid and glycocoll (Schmiedeberg). Again, Jacoby has shown that the liver will yield an extract capable of splitting off ammonia from urea, and that the same gland contains a ferment capable of transforming amido-acids into amides. Moreover, the liver and indeed the kidneys as well have the power of splitting up amides; a fact of the utmost importance in intermediary metabolism. Lastly, glycolytic action, in which both cleavage and oxidation of sugars may be involved, is likewise a function of many cells, and adds another striking illustration of intra-cellular ferment action, by which carbohydrate metabolism may be carried forward in the different tissues and organs of the body.

Oxidation is preëminently one of Nature's ways of bringing about alteration and decomposition, and in intermediary metabolism especially, oxidative processes must be quite conspicuous. Yet to-day we have accumulated a mass of evidence tending to show that oxidation in the tissues is

due primarily to the presence and action of a row of more or less closely related though chemically distinct ferments, known collectively as oxidases. Physiological oxidation therefore, as it occurs in metabolism, is likewise a result of intra-cellular ferment action. To be sure, the blood is the carrier to the tissue cells of the necessary oxygen, but the process of oxidation, with such chemical reactions as is involved, is due to the action of the intra-cellular oxidases. It is well to recall that Schönbein originally drew attention to the fact that the blood, and tissues as well, had the power when fresh of splitting off oxygen from hydrogen peroxide, but lost the power when heated to 90–100°. This phenomenon, however, is not, as was at one time thought, simply a general ferment indicator, but is due to the action of specific oxidases, some of which can actually be precipitated from the fluids containing them, and moreover differ among themselves as to the degree in which they are distributed among the different tissues and organs of the body. The spleen, liver, pancreas, thymus, muscle, brain and ovaries are noticeably rich in oxidizing ferment. In fact, the physiologist to-day has knowledge of a large number of intra-cellular oxidases, more or less distinct from each other, to some of which distinct names have been given indicative of their special lines of activity, viz., *aldehydase*, a ferment which oxidizes aldehydes to their corresponding acids, and present in the liver, kidneys, testicles, suprarenals, brain, lungs, thymus and salivary glands; *tyrosinase*, a ferment which oxidizes benzol derivatives, especially tyrosin; and *indolphenol-oxidase*, a corresponding ferment present in the pancreas, thymus, spleen, and salivary glands, but absent from the muscle and kidneys.

As can easily be imagined, these facts throw new light upon the methods of intermediary metabolism. They show us that the individual gland and tissue cells are provided

with efficient agents for accomplishing chemical changes of great variety. We are no longer forced to think or speak in vague terms of the somewhat mystical changes that go on in living tissues, but we have acquired sufficient knowledge to understand that the individual cells of the body are provided with intra-cellular ferments of definite character by which the metabolic changes are produced. These newly discovered enzymes are of such widely divergent nature and so broadly distributed among the tissues of the body that they offer an adequate explanation of the many chemical changes that occur in ordinary metabolic processes. Further, when it is remembered that practically all tissue cells contain proteolytic enzymes capable of transforming their own proteids into simpler products, and that in addition they have special enzymes endowed with a variety of powers, we see opening before us simple chemical methods of tracing out the individual steps in the gradual breaking down of the complex molecules of tissue material into the relatively simple bodies which we classify as intermediate and end products of tissue metabolism.

Let us now consider more in detail, and from the standpoint of what has already been said, some specific examples of intermediary metabolism. By the older methods of investigation, the physiologist was compelled to rely mainly for information upon such data as could be obtained by careful study of the urine, reinforced by examination of the individual glands and tissues. The detection in the latter of specific products led to the inference that these substances were formed in the glands under examination, but it was purely a question of inference as to how and from what the bodies originated. Further, if the urine showed the absence of these same bodies, then we naturally inferred that they were either completely destroyed or transformed into some other body, the methods of which were likewise enigmatical. The gaps and guesses of Foster were met with

at every turn in any attempt to trace out systematically the changes of intermediary metabolism.

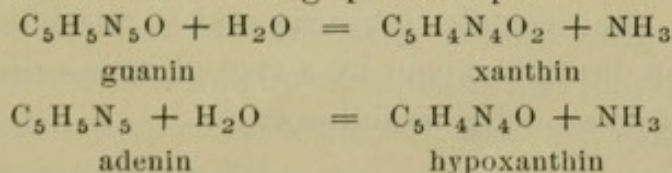
Modern methods and recent acquirements in knowledge are both well illustrated in what has been accomplished in the study of purin metabolism. Nucleoproteids of various kinds are conspicuous constituents of all cells; they are found in all tissues, in all glandular organs, and their widespread distribution may be taken as evidence of their great physiological importance. Their metabolism must of necessity be a conspicuous feature in the changes taking place in all glandular organs. Chemical study has shown that nucleoproteids by simple hydrolysis with mineral acids in a flask can be broken down into some form of proteid, phosphoric acid and one or more purin bases, such as adenin, guanin, xanthin and hypoxanthin. Further, chemical constitution would lead to the inference, verified by repeated experiment, that these purin bodies can be readily converted into uric acid. Do such reactions take place in the body; and, if so, by what agencies are they brought about?

If such glands as the thymus, suprarenal, spleen, etc., are subjected for some time to self-digestion at body-temperature in the presence of an antiseptic, it can readily be demonstrated that certain chemical changes occur. Thus, in the case of the thymus, large amounts of xanthin and a small amount of hypoxanthin, together with uracil, are found in the fluid. With the suprarenal, large quantities of xanthin and a small quantity of hypoxanthin are found.* With both of these glands, however, guanin and adenin are entirely lacking. In the self-digestion of the spleen, on the other hand, guanin is formed abundantly, also hypoxanthin, while adenin and xanthin are wanting. These results are quite different from what is found when the glands or their respective nucleoproteids are boiled with acid. In other words, when mineral acids are used as the hydro-

* Walter Jones: Ueber die Selbstverdauung von Nucleoproteiden. Zeitschrift für physiologische Chemie, Band 42, p. 34, 1904.

lyzing agents, then the tissue nucleoproteids yield, as a rule, guanin and adenin, but by autolysis xanthin and hypoxanthin are the conspicuous bases.* This difference in the result by hydrolysis with acid and by autolysis is due to the fact that in autolysis the changes induced are owing to the presence of specific intra-cellular ferments,† which have the power of acting upon certain of the purin bodies transforming them into other related substances.

Thus, in the self-digestion of the pancreas in an alkaline medium large amounts of xanthin and hypoxanthin are found as the end products of the autolysis. Guanin and adenin are no doubt also formed, but they are gradually converted into xanthin and hypoxanthin by intra-cellular ferments. If pure guanin itself is placed in a mixture containing finely divided pancreas, with chloroform to prevent putrefaction, and the mixture kept at 40°C. for some time, the guanin is slowly but surely converted into xanthin, until in time it is wholly replaced by the latter base. The ferment or enzyme that brings about this transformation is called *guanase*,‡ and is likewise present in the thymus and adrenals, but is absent from the spleen. A corresponding intra-cellular enzyme, known as *adenase*, and which transforms adenin into hypoxanthin, is likewise present in the thymus, adrenals, pancreas and liver.§ These two ferments are true hydrolyzing agents, the chemical reactions involved being quite simple:



In both of these cases it is to be noted that there is not only a taking on of water, under the influence of the enzyme,

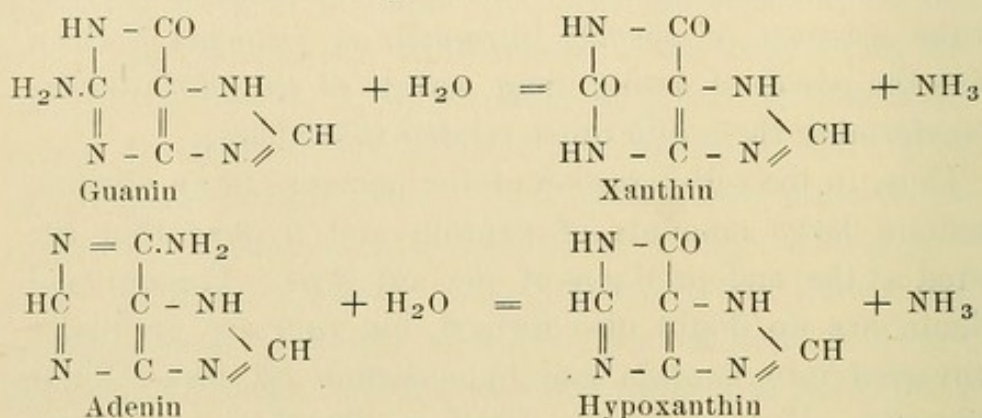
* Compare Levene: The Autolysis of Animal Organs. American Journal of Physiology, Vol. 12, p. 276, 1904.

† Jones and Partridge: Ueber die Guanase. Zeitschrift für physiologische Chemie, Band 42, p. 343, 1904.

‡ Jones and Partridge: Loc. cit.

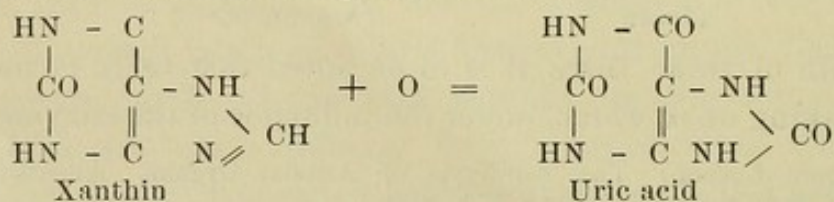
§ See Jones and Winternitz: Ibid., Band 44, p. 1.

with a retention of the oxygen, but there is also a giving off of ammonia, by which the transformation is made possible. The real essence of the reaction is best shown by use of the constitutional formulæ, whereby can be seen the full extent of the change which is effected:



Expressed in different language, these enzymes are able to transform the two amino-purins into the corresponding oxypurins xanthin and hypoxanthin; *i. e.*, they are typical deamidizing ferments, destroying the NH_2 group of the adenin and guanin.

Again, it is possible, as Schittenhelm* has shown, to prepare from the spleen simple extracts, free from all tissue elements, that are capable of transforming these purin bodies into uric acid. From such extracts, the enzyme which is the active agent in the oxidation can be separated by simple chemical means, and the purin bases can be transformed quantitatively into uric acid by its action. The conversion of xanthin, for example, into uric acid is purely a process of oxidation brought about by a typical intra-cellular oxidase, the reaction being as follows:



* Ueber die Ferments des Nucleinstoffwechsels. Zeitschrift f. physiologische Chemie, Band 43, p. 228. See also Schittenhelm: Ueber die Harnsäurebildung in Gewebsauszügen. Ibid., Band 42, p. 250.

Schittenhelm* has already demonstrated that this oxidizing enzyme is present in the liver, spleen, lungs and muscle, but is absent from the thymus, intestine, blood and kidneys, and Burian has given to the ferment the name of "xanthinoxidase."

Another interesting fact to be brought forward in this connection is the existence of a specific intra-cellular enzyme appropriately termed *nuclease*, which has the power of liberating the purin bases from their combination as a component part of tissue nucleoproteids, or more specifically of the contained nucleic acids. It has long been known that nucleoproteids, nucleins and nucleic acid when fed cause at once an increased output of uric acid, and it has been clearly recognized that uric acid as a product of metabolism must result from the transformation of the nuclein bases which are so conspicuous in the nucleins and nucleic acid; but as to how the uric acid was formed in the body could only be conjectured. Now, however, the matter is made quite clear, and we see how various intra-cellular enzymes working one after the other are able gradually to evolve uric acid from tissue nucleoproteids. Under the influence of a *nuclease*, nucleic acid is split up with liberation of the free nuclein bases, then by the action of a deamidizing enzyme guanin and adenin are transformed into xanthin and hypoxanthin respectively. Further, by the action of the oxidase just referred to, hypoxanthin is oxidized to xanthin, and the latter is converted into uric acid. Moreover, it is worthy of emphasis that these enzymes are not distributed indiscriminately, but are confined to specific organs or tissues, as has already been indicated. Finally, it is to be noted that there is another tissue oxidase — contained so far as is known at present in the kidneys, liver, muscle, and perhaps the marrow of bones (Schittenhelm) — which has the power of oxidizing and

* Loc. cit. Band 43, p. 236.

thus destroying uric acid. Here, then, we have four distinct enzymes or intra-cellular ferments, more or less responsible agents for the production and presence of uric acid in the body. The various steps in the intermediary metabolism, by which this substance results are made quite clear and we see how specific intra-cellular enzymes, formed by the tissue cells, are responsible for the chemical changes induced.

By all students of metabolism, the two-fold origin of uric acid — endogenous and exogenous — is well understood. It is equally well known that in the origin of uric acid from outside materials, free and combined purin compounds are equally effective; *i. e.*, nuclein-containing foods and nitrogenous extractives of the xanthin type. By the processes of metabolism, in which the intra-cellular enzymes referred to are the active agents, uric acid is formed, and eventually appears in the urine. It is evident, however, that exogenous uric acid cannot be controlled wholly by the character and quantity of the food consumed. To be sure, avoidance of purin-containing foods will necessarily result in a greatly diminished production of uric acid, but we must not overlook the part which the uric acid-oxidizing ferment plays in the destruction of this substance in the tissues of the body. As has already been pointed out, this peculiar oxidase has been found by Schittenhelm in the liver, muscle, kidneys, and perhaps in the bone marrow, and it is easy to see how the content of uric acid in the blood, lymph and urine may be determined as much by the relative activity of this oxidase, as by the activity of the nuclease, amidase and oxidase which produce uric acid from the purin-containing foods. It is perfectly clear that inhibition of the oxidizing action of the uric acid-destroying enzyme may be as effective in bringing about a high content of uric acid as increased production of uric acid. We have no means at present of judging the relative activity, under normal meta-

bolic conditions, of these two somewhat divergent factors, but I believe that the conspicuous increase of uric acid noticed under many conditions of life is due as much or more to increased inhibition of the oxidation of uric acid as to augmented production of uric acid. This is conspicuously true in the influence exerted by alcoholic drinks on the uric acid-content of the blood and urine.

Recent work carried on in the writer's laboratory* has shown quite conclusively that in man the ingestion of alcoholic fluids with purin-containing foods increases at once the output of uric acid in the urine. Alcohol, however, does not produce this result when taken with a light diet or one free from purin compounds. In other words, alcohol influences only the uric acid of exogenous origin, in conformity with the well-known gouty tendencies of high proteid feeding combined with consumption of alcoholic fluids. Alcohol is well understood to interfere with the oxidative processes of the liver, and it is more than probable that its influence on the uric acid content of the blood lies in the direction of inhibiting the action of the oxidase which, present in the liver and other tissues, normally destroys or oxidizes a certain proportion of the uric acid formed. This being true, we see another illustration of so-called perverted metabolism due entirely to a change in the rate of action of an intra-cellular oxidase.

In any reference to the origin of uric acid in the body, too much stress cannot be laid upon the easy convertibility of the *free* purin bases adenin, guanin, hypoxanthin and xanthin into uric acid,† by virtue of the action of the intra-cellular enzymes present in so many of the organs and tissues. In the ordinary foodstuffs, however, as in meat

* S. P. Beebe: The Effect of Alcohol and Alcoholic Fluids upon the Excretion of Uric Acid in Man. *Americal Journal of Physiology*, vol. 12, p. 13.

† See Krüger and Schmid: Die Entstehung der Harnsäure aus freien Purinbasen. *Zeitschrift f. physiologische Chemie*, Band 34, p. 549.

broth, it is the oxypurins hypoxanthin and xanthin that the body has to deal with mainly. These are quickly oxidized to uric acid which may then be excreted, or a portion may undergo oxidation to urea or other products. When, on the other hand, combined purins are introduced, as in the nucleins and nucleoproteids, adenin and guanin are liberated by the action of nuclease. These bodies are amino-purins, and their continuance unchanged depends upon the presence and action of the two enzymes adenase and guanase. If the latter are present and active in normal degree, we can conceive of a ready conversion into hypoxanthin and xanthin and then into uric acid. But if these enzymes are lacking or are inhibited in their action, then the two amino-purins will float about unaltered for a time at least. May we not find in this possibility an explanation for certain disturbed conditions of the body, especially of the kidneys, since experiments have shown that adenin in the case of dogs,* and in rabbits† when larger doses are given, is liable to produce anatomical alterations in the kidneys, particularly of the tubules, accompanied by peculiar deposits in the kidney tissue of spheroliths of uric acid and ammonium urate, which evidently cause irritation and marked alterations of structure. Here, again, we see suggested a threatened perversion of metabolism through possible absence or inhibition of an intra-cellular enzyme.

Uric acid of endogenous origin is as much a product of enzyme action as that which is derived from the purin compounds of the ingested food. It is well known that the elimination of uric acid does not cease during fasting, or with a diet free from purin compounds. The amount, however, is greatly reduced when purin-containing foods

* Minkowski: Untersuchungen zur Physiologie und Pathologie der Harnsäure bei Säugethieren. Archiv f. exper. Pathol. und Pharm., Band 41, p. 375.

† Schittenhelm: Das Verhalten von Adenin und Guanin im thierischen Organismus. Ibid., Band 47, p. 432.

are excluded from the diet. Many investigators* have shown experimentally that there is a certain degree of constancy in the output of uric acid on a non-purin diet, even when there are wide variations in the quality and quantity of the purin-free food. Various careful experiments, covering fairly long periods of time, carried on in our laboratory by Dr. E. W. Rockwood† on men taking only purin-free food, such as milk, wheat bread, butter, cheese, cereal, fruit, etc., showed a daily output of uric acid averaging 0.3—0.4 gram. Further, it was observed, in conformity with the thesis of Burian and Schur, that a given individual shows a certain degree of constancy in the daily excretion of uric acid. In other words, the elimination of endogenous uric acid is constant for each individual; *i. e.*, it is an individual factor dependent perhaps in part upon the weight of the body or the contained tissues and organs. It was a noticeable fact that the results obtained were not influenced in any measurable degree by the extent of muscular activity.

As to the origin of this endogenous uric acid, it has long been taken for granted that it must come in part from the breaking down or metabolism of the nucleoproteids of the tissue cells, by the action of the same agencies that are effective in the formation of exogenous uric acid. There are, however, other possible methods not to be entirely overlooked; viz., the possibility of a synthetical production of the acid in the liver or elsewhere, and also the possibility of some other antecedent than the nucleoproteids of the glandular organs, leucocytes, etc., serving as the direct mother substance in its formation. Knowing the fate of the nucleoproteids of the food and the marked influence

* Burian and Schur: *Archiv. f. die gesammte Physiologie*, Band 80, p. 241; also, Band 87, p. 239.

Sivén: *Skandinavisches Archiv für Physiologie*, Band 11, p. 132.

Wiener: *Ergebnisse der Physiologie*, 1902, i, Part I., p. 355; *Ibid.*, 1903, ii, Part I., p. 377.

† The Elimination of Endogenous Uric Acid. *American Journal of Physiology*, vol. 12, p. 38.

these exert upon the elimination of uric acid, reinforced by the abundant experimental evidence that both free and combined purin bases when ingested are transformed into uric acid, there has been little question that the endogenous uric acid must be derived from the breaking down of the nucleoproteids of the tissue cells. Indeed, there has been some disposition to take the amount of endogenous uric acid eliminated as a measure of the chemical activity or disintegration of tissue elements. This view, however, has quite recently received a severe shock in the results of some experiments recorded by Burian,* in which he shows quite conclusively that only a very small amount of the endogenous uric acid has its origin in the nucleoproteids of disintegrating tissue cells or leucocytes, the larger part coming from quite a different source, viz., from the purin base hypoxanthin, which is continually being formed as a metabolic product of living muscle tissues. Still, as already stated, muscular work, which naturally means increased muscular metabolism, has not appeared to cause any measurable increase in the output of uric acid. This may be true, according to Burian, with the 24 hours' urine, but it is not the case when the urine is examined in hourly periods, for he finds that after vigorous muscular exercise in the case of fasting individuals, where the hourly excretion of uric acid is fairly constant, there is a marked increase in the output of uric acid through the urine, the maximum excretion showing itself in the second hour after cessation of the work, enduring for an hour or longer, then slowly sinking below the normal. This fall in the rate of excretion thus balances in a measure the temporary increase, and explains why examination of the 24 hours' urine has failed to afford evidence of this peculiar influence.

This observation of Burian renders probable an intimate

* Die Herkunft der endogenen Harnpurine bei Mensch und Säugetier. Zeitschrift f. physiologische Chemie, Band 43, p. 532.

relationship between muscle metabolism, the formation or liberation of a purin base in the muscle, and its consequent conversion into uric acid by the intra-cellular xanthinoxidase. Further, the same investigator has shown that by perfusing a mixture of Ringer's solution and fresh dog's blood through the hind leg of a recently killed dog, the muscle fibres being maintained in a state of rest, the blood will gradually show the presence of uric acid; while if the muscles are stimulated by an induction current for an hour or so, then the circulating blood shows an increase of purin material, and the living muscle tissue itself shows an increased content of hypoxanthin during the period of "work." It is obvious from these statements that the muscle or the blood must contain xanthinoxidase to convert the liberated oxypurin into uric acid. This is indeed true of the muscle, for an extract of dog's muscle, like an extract of the liver or spleen, will transform to uric acid any oxypurin added, thus affording satisfactory proof that the muscle contains a specific intra-cellular oxidase. Further, there is evidence that the muscle also possesses the power of decomposing uric acid.

There is thus opened up a new chapter in purin metabolism, bearing upon the endogenous production of uric acid. In the resting state, muscle is continually giving up to the blood a certain amount of uric acid formed at the expense of the hypoxanthin which originates within its own tissue. The oxidation of the purin base to uric acid is accomplished by the specific oxidase which the muscle itself contains, but, as Burian points out, this enzyme must be so located that the hypoxanthin is converted into uric acid just as it is passing from the muscle fibre into the blood or lymph, since muscle itself never contains any uric acid, only purin base. Further, it is to be understood that a certain amount of the uric acid is at once decomposed by the action of the other oxidizing ferment which destroys this acid. Finally, Burian calls attention to the fact that

since the hypoxanthin-content of the muscles, in spite of the continuous formation and withdrawal of uric acid, remains in general constant, it follows necessarily that muscles during rest are continually forming new hypoxanthin as a normal product of their own metabolism. During activity, the formation of hypoxanthin in muscle is accelerated, while at the same time there is a corresponding increase in the output of the purin body from the muscle, the action of the xanthinoxidase being doubtless stimulated by the local deficiency of oxygen concomitant with the increased muscular activity, and as a final result there is an increased production of uric acid. What proportion of the thus formed uric acid appears in the urine we have at present no definite information, for we do not know in what degree the formed acid is further decomposed.

Assuming the correctness of the views just expressed, it is evident that muscle tissue takes a new position in the body as a uric acid-producing center. The tissue, it is true, does not differ from other tissues or organs as a depot for the specific oxidase, but unlike the other tissues it is constantly producing, both in rest and in activity, an oxy-purin ready for direct transformation. In the other uric acid-producing centers, activity is more or less intermittent, being dependent primarily upon the advent in the food of free or combined purin bodies upon which the intra-cellular amidases and oxidases can act. Burian considers, on the strength of his experimental results, that endogenous uric acid owes its origin mainly to these metabolic changes in muscle, and that only a very small proportion of the endogenous acid is derived from the nucleoproteids of the disintegrating tissue cells. Again, experimental evidence* is strongly opposed to the view that there is any synthetical production of uric acid in the animal body; the only way

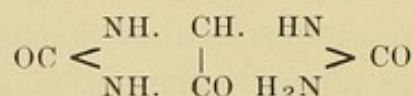
* Burian: Ueber die oxydative und die vermeintliche synthetische Bildung von Harnsäure in Rinderleberauszug. *Zeitschrift f. physiologische Chemie*, Band 43, p. 497.

at present known in which the exogenous uric acid can be increased in amount, being feeding with free purin bases, or with nucleoproteid-containing foods in which combined purin bodies are present. In other words, uric acid owes its origin entirely to processes of enzymatic oxidation.

We may advantageously pause here for a moment to consider the possible bearing of these facts upon so-called oxidation within the body. We have seen how in the processes discussed, oxidation is dependent upon specific ferments or enzymes, and we further understand that the intermediary products formed are definite bodies because of the specific nature of the active enzymes, and secondly, because of the chemical nature of the substances acted upon. In other words, oxidation in intermediary metabolism begins to take on the shape of a series of well-defined chemical processes in which chemical constitution and specific enzyme action are the predetermining causes. In the absence of the particular chemical groups, the oxidase is ineffective to bring about oxidation, or given the proper compound or mother substance in the absence of the specific oxidase there is no oxidation. Oxidation in the animal body, therefore, certainly as applied to intermediary metabolism, is no longer to be classed with ordinary combustion processes. We find, on the contrary, a series of orderly chemical processes, each one of which is presided over by an intra-cellular enzyme, specific in its nature in that it is capable of acting only upon bodies having a certain definite composition, and leading invariably to a certain definite result. Further, it is easy to see how perversion or inhibition of intermediary metabolism may be engendered by causes which act primarily upon the cells where the enzymes in question have their origin, or by influences which may bring about changes in the environment surrounding the intra-cellular ferment; for just as the ordinary extra-cellular digestive enzymes are influenced in their ac-

tivity by surrounding conditions, so may the intra-cellular oxidative enzymes feel the effect of changes in their environment.

In enzyme action in general, maximum results are obtained only when the enzyme and the substance to be acted upon bear a certain definite relationship to each other. A proportionate excess of one over the other may modify the rate of action, and where the substance undergoing change — in intermediary oxidation — is in relative excess over the enzyme there would seem suggested the possibility of a perversion of the normal oxidative change. In such a case as this, some intermediary body — perhaps abnormal in nature — might make its appearance. This principle in metabolism is well illustrated by some recent experiments carried on in our laboratory * bearing on the formation and excretion of allantoin in the animal body. Allantoin is an oxidation product of uric acid, having the formula :



It is found in the urine of pregnant women and in the urine of newly born infants. It is likewise excreted in large quantities by dogs after feeding pancreas, thymus, lymphatic glands, or any other material rich in nucleic acid. It also appears in the urine of the dog and cat after feeding relatively large amounts of uric acid. In other words, it is quite evident from the experimental records that in the case of these animals at least the purin bases (and nucleins) may give rise, under some conditions, to allantoin as an intermediate product of their oxidation. Ordinarily, these compounds of the purin type are more completely oxidized in the system, with formation of urea, although, as we have seen, a certain proportion of the nitrogen is excreted

* Mendel, Underhill and White, American Journal of Physiology, Vol. 8, p. 380. In this article are contained references to a large number of papers bearing on this subject.

as uric acid. But when larger quantities per kilo of body-weight of uric acid, or of the antecedent purin compounds, are introduced or formed in the system, the oxidative enzymes are unable to bring about as complete a decomposition of the purin radicles, and under such conditions allantoin escapes as an end-product of the incomplete oxidation of the acid.

Drugs that are known to influence certain lines of metabolic activity in which the purin bodies are concerned, may well have their action explained by assuming an influence upon enzymatic oxidation, as well as by attributing the effect to some general disturbance of functional activity of the gland or tissues. Further, with this mode of explanation at hand, it is easier to understand how drugs that bring about marked pathological conditions may still be free from influence upon some of the lines of functional activity with which the gland or tissue is associated. Thus, as some experiments recently carried on in our laboratory show,* phosphorus which produces such marked hepatic degeneration does not interfere in the case of the dog with the power to elaborate uric acid during a diet rich in purin compounds. In other words, the specific nuclease, amidase and xanthinoxidase are not inhibited in their action by the presence or influence of phosphorus, but uric acid is formed as under normal conditions when purin-containing foods are exhibited. Further, other experiments† made in our laboratory with sulphonal, a typical hepatic drug, tend to show that in fasting dogs there is no undue formation of allantoin when soluble urates are injected into the circulation, *i. e.*, the normal uric acid oxidation is not interfered with, even though the animal be strongly under the in-

* Mendel and Jackson: American Journal of Physiology, Vol. 4, p. 168.

† Mendel and White: On the Intermediary Metabolism of the Purin Bodies: The Production of Allantoin in the Animal Body. American Journal of Physiology, Vol. 12, p. 93.

fluence of the drug. When, however, animals fed on pancreas, *i. e.*, with an abundance of nucleoproteid, are treated with sulphonal, then it is found that allantoin, which on the above diet would normally appear in large amounts, is never present in the urine. Presumably, the sulphonal in some way influences favorably the oxidation of the purin compounds to uric acid and urea, and it is reasonable to assume that this action is due to some stimulating influence upon the oxidases of the liver or other glands, as to ascribe it to any other more general cause.

We must not, however, confine our attention too long to one particular line of intermediary metabolism, remembering that there are many other chapters in nutrition to which attention may profitably be directed, in illustration of our main theme. Let us therefore consider next some phases of the intermediary metabolism of carbohydrates and its perversions, which will afford us opportunity also to discuss the formation of oxalic acid, glycuronic acid, etc. It is well understood that under ordinary conditions of life and health, the carbohydrates of our food and body tissues are continually undergoing oxidation with ultimate formation of carbon dioxide and water, but it is not to be supposed that this oxidation necessarily takes place wholly at one step. In the blood, as Lépine* originally pointed out, there is present a glycolytic ferment or enzyme which has the power of destroying sugar, but as Krauss† and Arthus‡ have shown, this action of the blood is not sufficiently strong to bring about the oxidation of the several hundred grams of sugar that are being broken down in the body each day. The discovery by v. Mering§ and Minkowski that extirpation of the pancreas produces a severe form of diabetes led

* Comptes rendu, Tome 110, p. 742 and p. 1314.

† Zeitschr. f. klin. Med., Band 21, p. 315.

‡ Arch. de Physiol., Tome 24, p. 337.

§ Diabetes mellitus nach Pankreasextirpation. Archiv f. exper. Pathol. u. Pharm., Band 26, p. 371.

naturally to the view that this organ normally produces something, possibly an internal secretion, failure of which renders the body unable to burn sugar. Search for the presence of a glycolytic ferment in the pancreas, however, gave negative results.* It remained for Cohnheim† to show that when the pancreas is combined with muscle there is developed a strong sugar-decomposing action. The pancreas alone has no such power, and muscle likewise when taken by itself shows no appreciable glycolytic action, but the two together have such power that 5.6 grams of sugar can be destroyed or burned by a kilogram of muscle. If, says Cohnheim, we estimate that an adult man possesses 40 kilograms of muscle, this tissue influenced by the action of the pancreas is quite capable of oxidizing over 200 grams of dextrose.

This oxidizing power is not dependent upon the presence of the tissue cells, but the active agent can be extracted and the resultant cell-free fluid shows the same power of oxidizing dextrose, thus signifying quite clearly that the agent is a soluble enzyme or ferment, which presumably resides in the muscle tissue in an antecedent form and is activated through some influence exerted by the pancreas. The action is thus analogous to the influence exerted by the *enterokinase* of the intestinal glands, which has the power of transforming inactive trypsinogen into the active enzyme trypsin, the proteolytic enzyme of the pancreatic juice. It is true that these results of Cohnheim have been adversely criticised by some investigators, notably by Stoklasa and by Claus and Embden, but nevertheless the general trend of the results seems pretty clearly established, and it appears evident that glycolytic action, which undoubt-

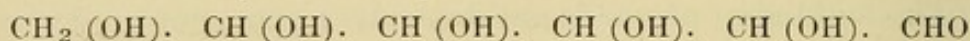
* Herzog: Liefert das Pankreas ein Dextrose spaltendes, alkohol und kohlensäure bildendes Enzym? Hofmeister's Beiträge zur chemischen Physiologie und Pathologie, Band 2, p. 102.

† Die Kohlehydrateverbrennung in den Muskeln und ihre Beeinflussung durch das Pankreas. Zeitschrift für physiologische Chemie, Band 39, p. 336. Also, Band 42, p. 401, and Band 43, p. 547.

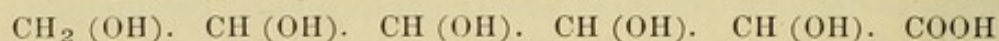
edly occurs in most, if not all, of the active tissues of the body, is due to soluble enzymes, the activity of which is heightened through the influence of some agent or agents furnished by the pancreas.

In this oxidation of sugar by the tissues of the body, a large proportion of the sugar burned is unquestionably entirely destroyed, but not always is the oxidation complete, and then intermediate products make their appearance as a sign and a warning of physiological processes gone astray. Incomplete or improper burning of sugar may — in the absence of any better explanation — be ascribed to lack of the specific enzyme or enzymes, or to lack of suitable conditions for their proper activation.

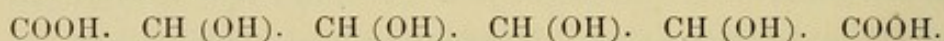
The sugar of the blood, it will be remembered, is dextrose, an aldehyde, having the formula :



By mild oxidation, the aldehyde group (CHO) is changed into an acid group (carboxyl group, COOH) and monobasic gluconic acid results, viz. :



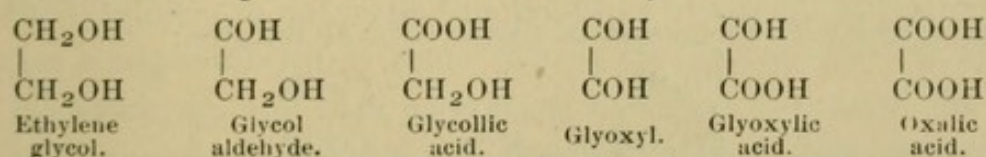
By more energetic oxidation, the dibasic saccharic acid is formed, viz. :



These two illustrations suffice to show the readiness with which aldehyde groups (CHO) and primary alcohol groups (CH_2OH) are oxidized to acid groups (COOH). It might be assumed therefore that all substances containing the above groups would undergo oxidation in the body along the lines indicated, just as ordinary alcohol when introduced into the system may yield aldehyde and acetic acid when the oxidation is somewhat restricted. As we have tried to point out, however, oxidation in the animal body is something more than a mere exposure to oxygen, and it does not necessarily follow that all substances con-

taining these groups will be oxidized in rhythmic fashion in the body, or through a regular series of successive stages. The very fact that such results do not always occur affords the best of evidence that animal oxidation involves other factors than simple oxygen; *i. e.*, is in harmony with the view that oxidation of a given substance depends in large measure upon the presence of specific intra-cellular oxidases which can attack that particular substance. This view is forcibly illustrated by the work of Paul Meyer* with ethylene alcohol, or glycol, and its derivatives.

The oxidation products of glycol that would naturally come under physiological consideration are the following, the relationship of which is indicated by the formulæ:



It has been found by Pohl† that glycol or ethylene alcohol in the case of the dog can be only partially burned in the body, and that its introduction always gives rise to a large production of oxalic acid. Mayer has found the same to be true in the case of rabbits. It is a noticeable fact, however, as pointed out by Mayer, that when glycol or ethylene alcohol is given to rabbits in moderately large doses, there is a large output of glycollic acid. In fact, it may be stated that under the above conditions about one-fourth of the glycol is oxidized simply to glycollic acid, while the presence of considerable oxalate in the urine demonstrates further oxidation of a portion of the glycol to oxalic acid. With sufficient dosage of glycol, rabbits succumb with severe hæmorrhagic nephritis, the glomeruli being practically occluded with crystals of calcium oxalate

* Experimentelle Beiträge zur Frage des intermediären Stoffwechsels der Kohlehydrate. Zeitschrift f. physiologische Chemie, Band 38, p. 135.

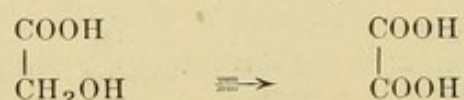
† Ueber den oxidativen Abbau der Fettkörper im thierischen Organismus. Archiv f. exper. Pathol. u. Pharm., Band 37.

and the whole section of the kidney tissue showing large masses of this crystalline salt.

The significance of these facts in the present connection lies in the probability, as suggested by Mayer, that this peculiar oxidation by which glycollic and oxalic acids result without any appearance of bodies intermediate between these two, is due first to simple oxidation of the primary alcohol group of glycol to the carboxyl group, thus giving rise to glycollic acid, as follows :



By further oxidation of the so-formed glycollic acid, the remaining primary alcohol group of the acid is similarly attacked with formation of oxalic acid as follows :



In other words, we have here a striking example of a definite line of oxidative action, involving an attack upon a distinct group of atoms, with a complete ignoring of other groups which *a priori* are seemingly just as easy of oxidation. How can this selective action be accounted for other than by the assumption that we are dealing with specific oxidases which have an affinity as it were for distinct groups or radicals, reinforced by an influence exerted by the stereochemical configuration of the molecule acted upon. In any event, these results are a striking refutation of the view that animal oxidation follows ordinary chemical and physical laws.

This statement may be reinforced by reference to the results obtained by Mayer* in his experiments with glycol aldehyde. This substance by oxidation would naturally give rise to glycollic acid, glyoxylic acid and oxalic acid, as suggested by the preceding formulæ. If, however,

* Loc. cit., p. 151.

glycol aldehyde is administered subcutaneously to rabbits, it is at once oxidized without any formation of bodies intermediate between the aldehyde and oxalic acid; glycollic acid and glyoxylic acid, which might be expected, are not found even in traces. On the other hand, dextrose is formed in considerable quantity. This formation of dextrose after injection of glycol aldehyde is not to be ascribed to increased formation of oxalic acid, for it is noticeable that excretion of the sugar commences within twenty minutes after the aldehyde is introduced and before any acid formation could have exerted much influence. More plausible is the hypothesis, suggested by Mayer, that a certain proportion of the glycol aldehyde is directly condensed in the organism to dextrose, the oxidative power of the organism being inhibited in some degree by the toxic action of the aldehyde, and thus permitting a portion at least of the so-formed dextrose to escape combustion and thus appear in the urine. So here again we find evidence of specific oxidative action in the organism, due no doubt to a specific aldehydase, combined with a process of condensation, both of which are very suggestive in connection with our understanding of the processes involved in intermediary carbohydrate metabolism.

The formation of oxalic acid in the body, just referred to in connection with the oxidation of glycol derivatives, is worthy of further consideration in connection with carbohydrate metabolism. The work of Mohr and Salomon* has shown that the body of man normally produces a small amount of oxalic acid which is excreted in the urine. With an oxalate-free diet, the daily urine contains 2-6 milligrams of the acid, increased somewhat by certain foods such as gelatin. Practically, all the tissues of the body contain small amounts of oxalate; muscle of man, for example,

*Untersuchungen zur Physiologie und Pathologie der Oxalsäurebildung und-Ausscheidung beim Menschen. Deutsch. Archiv. f. klin. Medizin, Band 70, p. 486.

showing 6.5 milligrams of oxalic acid per kilo of tissue.* According to Salkowski, the spleen, liver and muscle by autolysis in the presence of uric acid form a small amount of oxalic acid. More pertinent, however, are the observations of Hildebrant† who found on giving dextrose to rabbits fed on oats (which contain lime salts) that there was a very great increase in the production of oxalic acid, the acid appearing in the urine as calcium oxalate. In other words, under some conditions at least, oxalic acid may be considered as an oxidation product of dextrose.

In this connection, it is to be remembered that many observations‡ tend to show that in the incomplete oxidation of sugar in the body, glycuronic acid is a conspicuous product. Thus, in diabetes mellitus there is frequently, if not most generally, an increased excretion of glycuronic acid; a condition which often persists even when in consequence of a strict anti-diabetic diet, sugar excretion has entirely disappeared. Moreover, there are many diseases in which sugar rarely appears in the urine where glycuronic acid is present in increased quantity. Thus, as stated by Mayer, there are various acute febrile conditions in which increased excretion of glycuronic acid is found in harmony with the fact that in these diseases the power of assimilation for dextrose is inhibited somewhat, coupled with diminished power of oxidation of sugar, thereby resulting in a portion of the dextrose being oxidized only to glycuronic acid, while the conditions do not admit of any excretion of unchanged sugar. Further, it is to be noted that when a soluble salt of glycuronic acid is given to rabbits there results a marked increase in the output of oxalic acid, proportional to the amount of glycuronate fed. In diabetes, oxaluria is occa-

* Cipollina: Ueber die Oxalsäure im Organismus. Berliner klin. Wochenschrift, 1901, p. 544.

† Uber eine experimentelle Stoffwechselabnormität. Zeitschrift f. physiologische Chemie, Band 35, p. 141.

‡ See especially Paul Mayer: Uber unvollkommene Zuckeroxydation im Organismus. Deutsche med. Wochenschrift, 1901, p. 243 and p. 262.

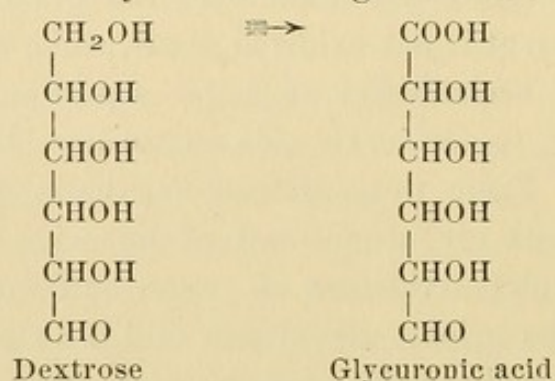
sionally met with, in harmony with a preceding statement that oxalic acid is liable to result from the incomplete combustion of dextrose. The well known antagonism between glycosuria and oxaluria frequently observed is due to the fact that in severe cases of diabetes the greater portion of the sugar present is not oxidized at all, while with improvement in the assimilation of sugar a portion is oxidized, not, however, to carbon dioxide and water, but simply to oxalic acid. From these statements, the inference may be drawn that both glycuronic and oxalic acids are products of the incomplete oxidation of sugar, and further, that in some instances at least glycuronic acid is an antecedent or intermediary body in the production of oxalic acid.

Glycuronic acid is unquestionably an important body in the intermediary metabolism of carbohydrates. It is much more widely distributed and much more common than has generally been supposed. It does not occur in the free state in the animal body, but exists conjugated with various aromatic substances, notably with phenol, indoxyl, etc., these conjugated forms being normally present in the urine. The introduction of camphor or chloral hydrate (with which glycuronic acid combines) into the system is followed by a marked increase in the excretion of glycuronic compounds in the urine, thus implying the formation in the body of much more glycuronic acid than is indicated ordinarily by the content of this substance in the urine.

As to the origin of glycuronic acid, there is abundant experimental evidence, in harmony with what has previously been stated, that in rabbits at least the acid can be formed from dextrose. Further, there is likewise experimental evidence tending to show that in all probability glycuronic acid can also originate from proteid, *i. e.*, from the carbohydrate group of the proteid molecule.* The transfor-

* See Paul Mayer: Neue Untersuchungen über die Glukuronsäure. Biochemisches Centralblatt, Band 1, p. 377.

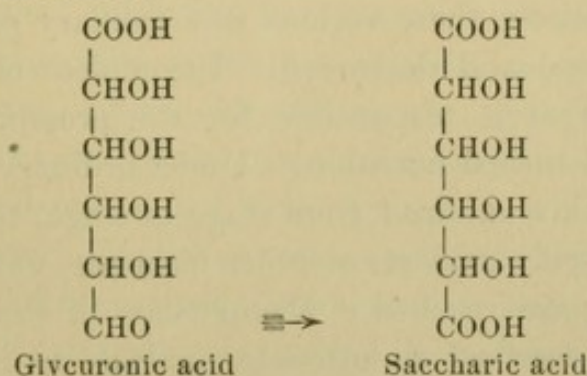
mation which dextrose undergoes when oxidized to glycuronic acid is very slight, and consists simply in the conversion of the primary alcohol group of the sugar into the carboxyl group of the acid, leaving the aldehyde group of the sugar intact, as indicated by the following formulæ:



A priori, it might be expected that in the foregoing oxidation the very labile aldehyde groupe would be the one to undergo change with formation of the monobasic gluconic acid, but the facts available do not support this view and the conclusion may be accepted as affording another evidence of the specific action of tissue oxidation, in which without doubt intra-cellular enzymes are the active agents. The evidence at hand, both experimental and clinical, renders quite conclusive the view that glycuronic acid appears in the tissues and in the excretions as the result of an incomplete oxidation of dextrose (both that which is derived from carbohydrate and that which has its origin in the breaking down of the proteid molecule), and consequently the amount of this acid present may be taken as a measure of the extent to which the sugar is escaping oxidation or combustion. Further, it is to be understood that glycuronic acid is a normal intermediary product of carbohydrate metabolism, representing perhaps the first step in the breaking down of sugar, which under ordinary or normal conditions progresses to further stages with complete or nearly complete disappearance of the intermediary glycuronic acid, except in those cases where further oxida-

tion is wholly or in part inhibited. In the latter case, the amount of glycuronic acid in the circulation is greatly increased and so likewise the various aromatic substances, which under ordinary circumstances are combined with it in small degree, are conjugated in larger quantities, thereby leading to an increased excretion of phenol, indoxyl and other glycuronates, with a corresponding decrease in the excretion of ethereal sulphates.

If glycuronic acid represents the first stage in the intermediary metabolism of sugar, then the dibasic saccharic acid might well be suspected as the second product in a progressive oxidation, where the aldehyde group of glycuronic acid is replaced by the carboxyl group as indicated in the following formulæ, a view which is supported by experimental evidence :



Comparison of the structural formula of saccharic acid with that of oxalic acid $\begin{array}{c} \text{COOH} \\ | \\ \text{COOH} \end{array}$ shows how easily by one

or more changes the latter acid may be formed by progressive oxidation, in harmony with the fact already mentioned that administration of glycuronic acid is followed by an increased excretion of oxalic acid, accompanied by accumulation of oxalic acid in the liver. The liver is unquestionably one of the places where glycuronic acid is oxidized to oxalic acid, since it is easy to demonstrate by digestion experiments with comminuted liver tissue the transformation of the former acid to oxalic acid; an observation of

obvious importance in intermediary metabolism. Finally, it may be mentioned that there is some ground for the belief that a part of the dextrose in the body is normally oxidized without first passing through the intermediary stage of glycuronic acid. How large this fraction may be cannot be said, but in any event the glycuronic acid that is formed is further oxidized with subsequent formation of a variable amount of oxalic acid.

As we study these various intermediary steps and stages in carbohydrate metabolism, we cannot avoid being impressed more and more fully with the general principle that the appearance of the so-called abnormal products of carbohydrate degradation, like sugar itself, is due not so much to perverted metabolic action as to inhibition of one or more of the normal oxidative processes by which, under normal conditions, these various intermediary products are further oxidized and destroyed. Incomplete or improper burning of sugar is responsible for the presence of these symbols of disturbed nutrition. Under ordinary conditions they are quickly oxidized from stage to stage, through the agency of specific oxidases or other ferments, until the final end-products are reached. Disturbance of function may therefore be ascribed in ultimate analysis to lack of the specific enzyme, or to lack of suitable conditions for the proper action of the enzyme. The burning of sugar in the body is as much dependent upon the progressive action of specific oxidizing enzymes as the hydrolysis of carbohydrates in general is dependent upon the action of amylolytic enzymes. This principle is well illustrated by some experiments recently carried on in our laboratory by Mr. P. H. Mitchell, in which it was found, for example, that soluble erythrodextrin when injected subcutaneously or intraperitoneally was thrown out through the urine in large amount as achroodextrin; *i. e.*, essentially unchanged. Though readily oxidizable, by this method of introduction the dex-

trin escaped contact with the enzymes which under other conditions would have quickly oxidized and destroyed it. Similarly, Mr. Mitchell found that the carbohydrate inulin introduced in like manner could be recovered in the urine to the extent of 70 per cent. wholly unchanged. Dr. Paul Mayer has reported a similar experience with glycogen, recovering 50 per cent. of the carbohydrate essentially unchanged in the urine after subcutaneous injection. In other words, the specific enzymes, the intra-cellular ferments of the tissues, as well as the amylolytic enzymes of the digestive juices, must have proper opportunity for action if the successive steps of carbohydrate metabolism or oxidation are to be successfully carried out.

Again, in many forms of experimental diabetes such, for example, as we have been in the habit of speaking of as due to "insults" offered to the pancreas, where such substances as piperidin, potassium cyanide, morphin, coniin, nicotin, curare, ether, chloroform, etc., produce marked hyperglycæmia and glycosuria, the explanation now offered* is that these drugs do not have any specific action upon any particular gland, like the pancreas, but that they exert more or less of an influence upon the respiratory centre, producing dyspnœa. Experiments conducted in our laboratory by Dr. Underhill have shown, for example, that while piperidin painted on the pancreas causes marked hyperglycæmia and consequent glycosuria, the same result follows when the piperidin is painted on the spleen, introduced intraperitoneally, or injected directly into the blood. Further, the drug fails to produce these results when oxygen is administered. In other words, as stated by Dr. Underhill, experimental diabetes of this sort is due simply to diminished oxidation of carbohydrate material, with the consequent accumulation of sugar in the blood, and elimination by the

* Underhill: Certain Aspects of Experimental Diabetes. American Journal of Physiology, Vol. 13, p. xxxvi.

kidneys. Dyspnœa is always prone to call forth marked hyperglycæmia and glycosuria, whether induced by drugs or otherwise. Lastly, may we not query whether the lack of oxidation of sugar and the consequent glycosuria in these cases is not due to conditions unfavorable or inhibitory to the action of the normal oxidases or other ferments which ordinarily are effective in destroying sugar?

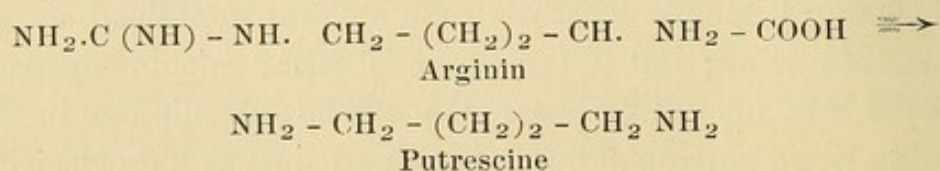
Another chapter in intermediary metabolism well worthy of consideration is that which relates to the amino-acids; but time will not permit of more than a passing reference to these interesting substances, although many of them are formed as products of proteid decomposition within the body, and play important parts in intermediary metabolism. Some of them, however, are connected with peculiar anomalies of proteid katabolism, and I may therefore be pardoned if I refer to one or two, since they are associated with conditions of the body in which certain metabolic functions are strangely perturbed. Cystin (and the related body, cystein) is well known as a primary crystalline decomposition product of proteids and is characterized by containing the sulphur of the proteid molecule from which it is derived. In that somewhat rare disease, known as cystinuria, cystin appears in the urine, sometimes in fairly large amounts. Normally, the cystin which must be split off from every sulphur-containing proteid during the process of katabolism is oxidized or destroyed by the processes of intermediary katabolism and disappears. But in cystinuria, the body loses the power of burning or oxidizing this substance, and hence it is excreted unchanged. Now, it is claimed that there are two, isomeric, forms of cystein; one, known as protein-cystein, because of its production from horn and other proteids, and having the formula: $\text{CH}_2 \cdot \text{SH} - \text{CH} \cdot \text{NH}_2 \cdot \text{COOH}$, *i. e.*, α -amino- β -thio-propionic acid; while the other, known as calculus-cystein, because obtained from urinary calculi, has the formula:

$\text{CH}_2 \cdot \text{NH}_2 - \text{CH} \cdot \text{SH} - \text{COOH}$, *i. e.*, α -thio- β -amino-propionic acid. If the so-called protein-cystein is fed to a person afflicted with cystinuria the substance is excreted unchanged, simply increasing the amount contained in the patient's urine; but if the isomeric form, calculus-cystein, is fed it at once disappears, the amount of sulphate in the urine being correspondingly increased, just as happens with both forms when introduced into the system of the healthy individual.* In other words, under the conditions prevailing in cystinuria, the body has lost the power of burning the one form of the amino-acid, *i. e.*, the α -amino acid. Indeed, in this condition of disease, intermediary metabolism is so perverted that all α -monamino acids whenever introduced into the system are excreted unchanged, the body having apparently lost all power of burning them. Thus, the α -monamino acids, tyrosin, leucin, aspartic acid, etc., which are completely oxidized in the healthy organism to carbon dioxide and ammonia, *i. e.*, as uræa, are excreted almost quantitatively when fed in cystinuria. We may well pause here to emphasize the fine discrimination which the body shows in its treatment of these two isomeric forms of cystein (in cystinuria). The two substances are essentially alike, except in the relative position in the molecule of the amino (NH_2) group, yet the one is easily oxidized, while the other passes through the body absolutely unchanged. Moreover, it is evident that in cystinuria this inhibition of the power to burn the α -amino acid is a general one, preventing any α -amino acid from undergoing change in the body.

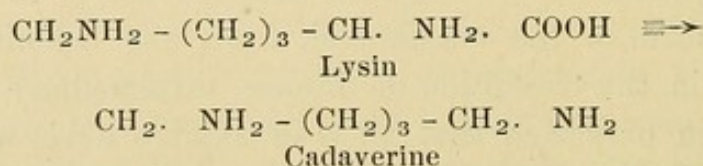
Further, in cystinuria, diamino-acids, such as arginin and lysin, behave in a peculiar manner. Unlike the monamino-acids, they undergo change, but it results simply in a slight transformation and not in a complete or

* See Loewy and Neuberg: Ueber Cystinurie. Zeitschrift f. physiologische Chemie, Band 43, p. 338.

profound oxidation. Thus, when arginin is fed to a person with cystinuria it is converted into the diamine putrescine, viz., tetramethylene-diamine :



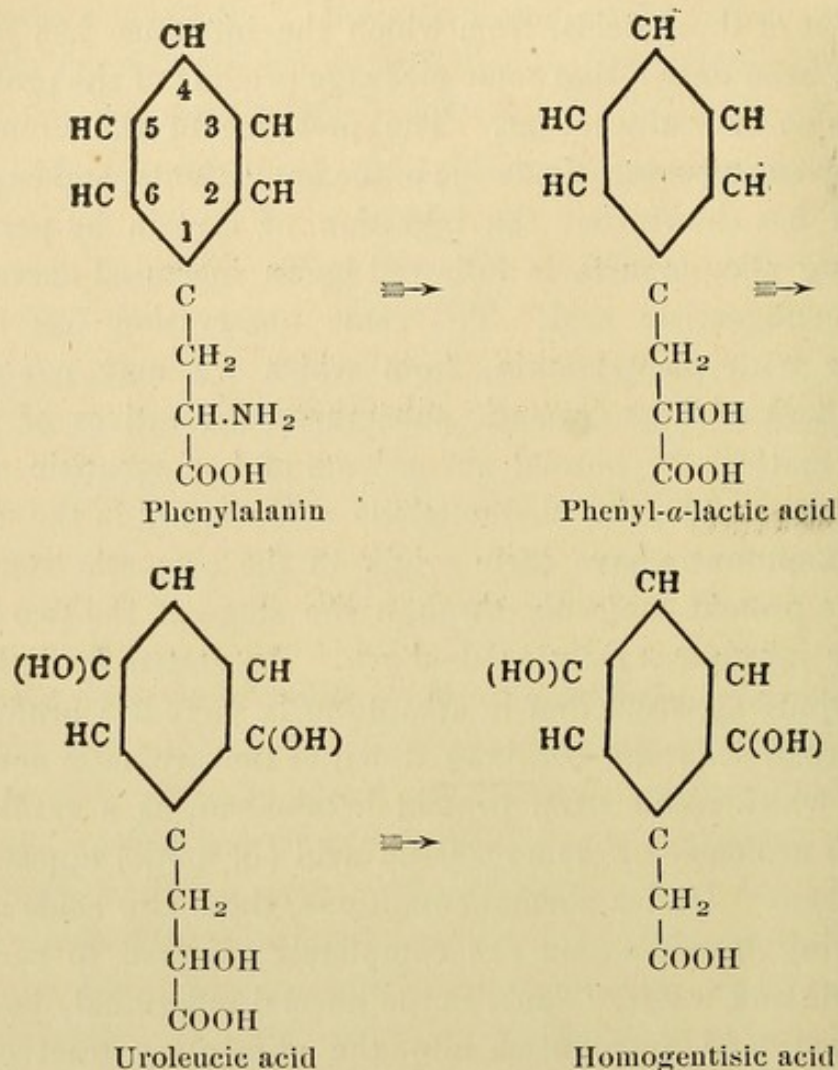
In a similar way the diamino-acid lysin is transformed into the diamine cadaverine, viz., pentamethylene-diamine :



With these facts before us as illustrations, it is evident that in such a disease as cystinuria many changes of metabolism may occur, by which intermediate products ordinarily undergoing progressive oxidation are prevented from suffering further change, and are hence cast out of the body unaltered. Their presence in undue quantities, however, is not without possible physiological significance. For example, it needs very little imagination to see how, when the body has lost in greater or less measure the power to burn organic acids, a condition of acidosis may be produced, and how this condition may in turn influence the production of ammonia in the body from the breaking down of proteid in an attempt on the part of the latter to neutralize or overcome the influence of the accumulating acid. But we have not time to discuss this theme, however important or suggestive it may be. Rather let me bring to your attention another anomaly of intermediary metabolism, as seen in alkaptonuria, a condition in which the urine shows the presence of the peculiar diphenol acids known as homogentisic and uroleucic acids. It has long been known that in this disease a rich proteid diet tends to increase the

output of these acids, from which the inference has naturally been drawn that some cleavage product of the proteids must be their antecedent. The presence of the aromatic group suggests tyrosin as one of the antecedents, and experiment has shown that the ingestion of tyrosin by persons having alkaptonuria is followed by an increased excretion of homogentisic acid. The same observation has been made with phenylalanin, from which we may conclude that both of these aromatic substances, derivatives of proteid matter, are normal antecedents of homogentisic acid. In other words, the characteristic acids found in the urine in alkaptonuria have their origin in the aromatic complex of the proteid molecule through the stage of the two aromatic substances mentioned above. As stated by Falta,* it is quite apparent that in alkaptonuria there is a profound disturbance in the breaking down of the aromatic amino-acids which result from proteid katabolism, as a result of which uroleucic or homogentisic acid (or both) appear in the urine. Under normal conditions, these two acids after suffering deamidization are completely oxidized to carbon dioxide and water. Thus, in the normal individual, homogentisic acid introduced into the alimentary tract completely disappears, *i. e.*, it is entirely oxidized to simpler products, but when fed to a person afflicted with alkaptonuria it appears quantitatively in the urine. In other words, in alkaptonuria the body has entirely lost the power of splitting off the benzol ring from the intermediary proteid product homogentisic acid. On the basis of experimental evidence, it is probable that the progressive breaking down of the aromatic amino-acids derived from proteid katabolism, after having reached the tyrosin or phenylalanin stage, is as follows :

* Die Alkaptonurie. Biochemisches Centralblatt, Band 3, p. 173.

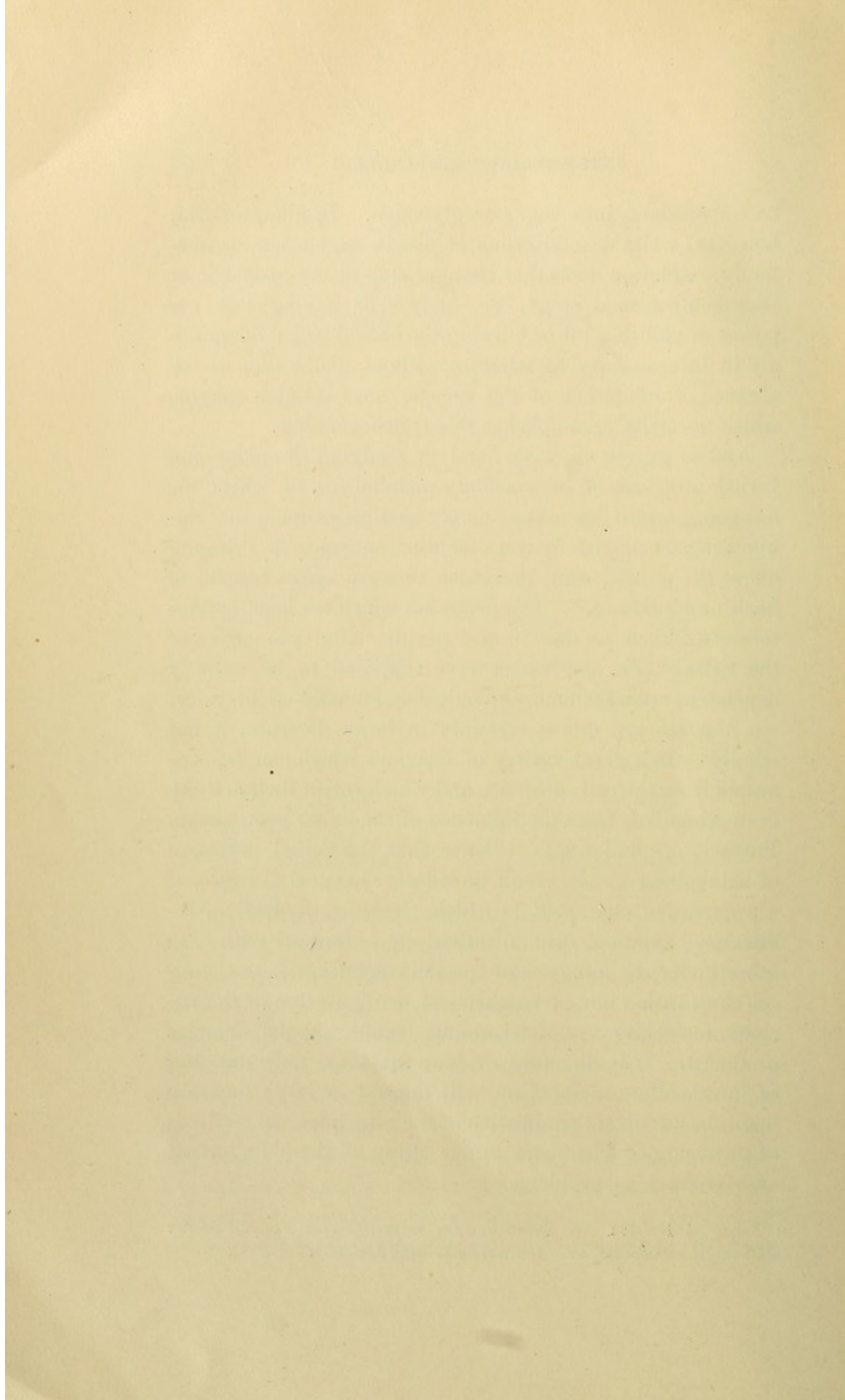


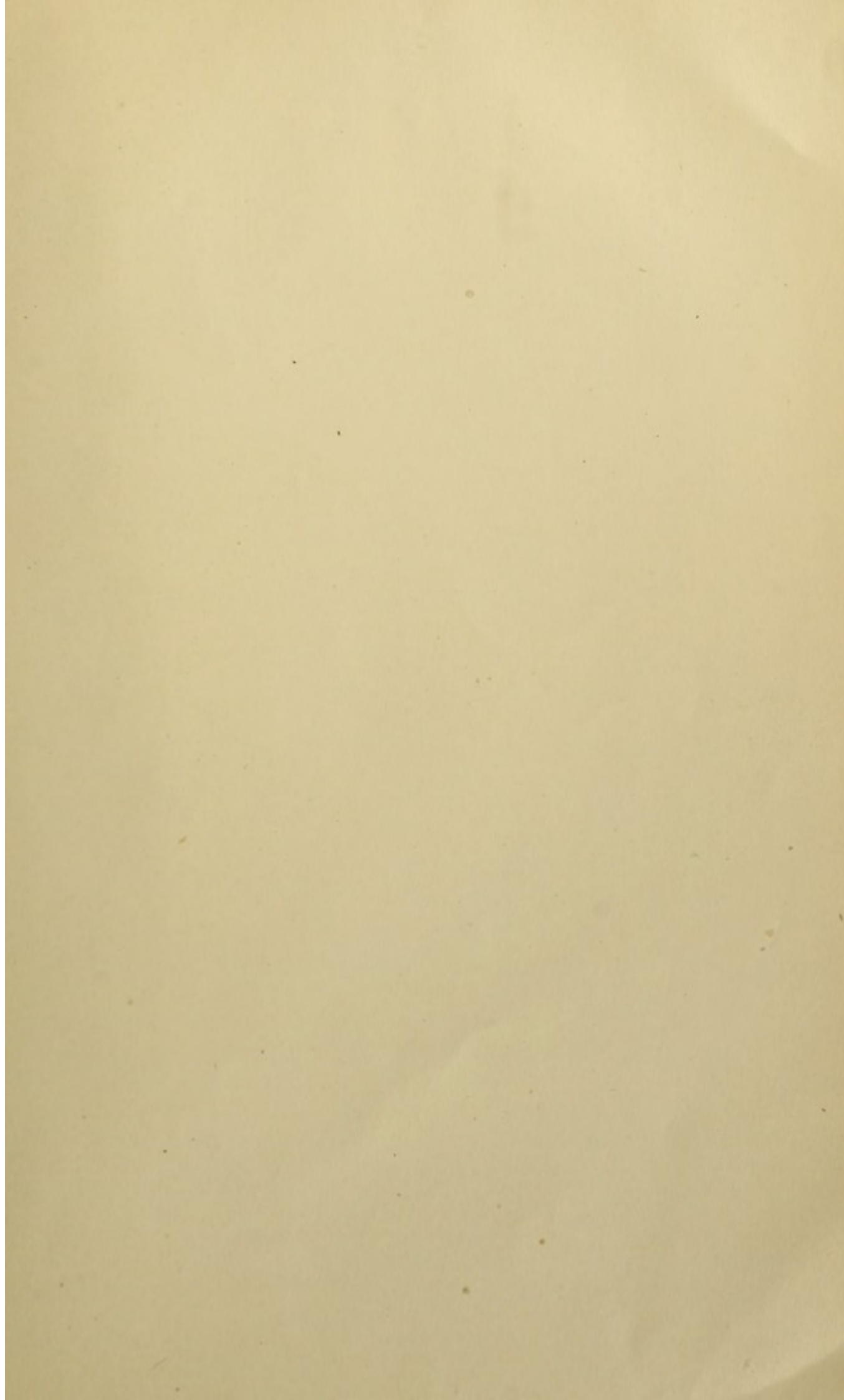
Especially interesting and suggestive in this schematic representation of the intermediary changes from phenylalanine to homogentisic acid is the formation of phenyl- α -lactic acid. The splitting off of nitrogen from the alanine, *i. e.*, its deamidization, takes place at this stage with the resultant formation of a non-nitrogenous acid, a complex of the aromatic group and α -lactic acid. We thus see opened up, as stated by Falta, a new point of view regarding the formation of sugar from proteid. Between lactic acid and dextrose there are many obvious physiological connections and it is quite conceivable, to say the least, that a portion of the lactic acid originating in this manner may in the liver

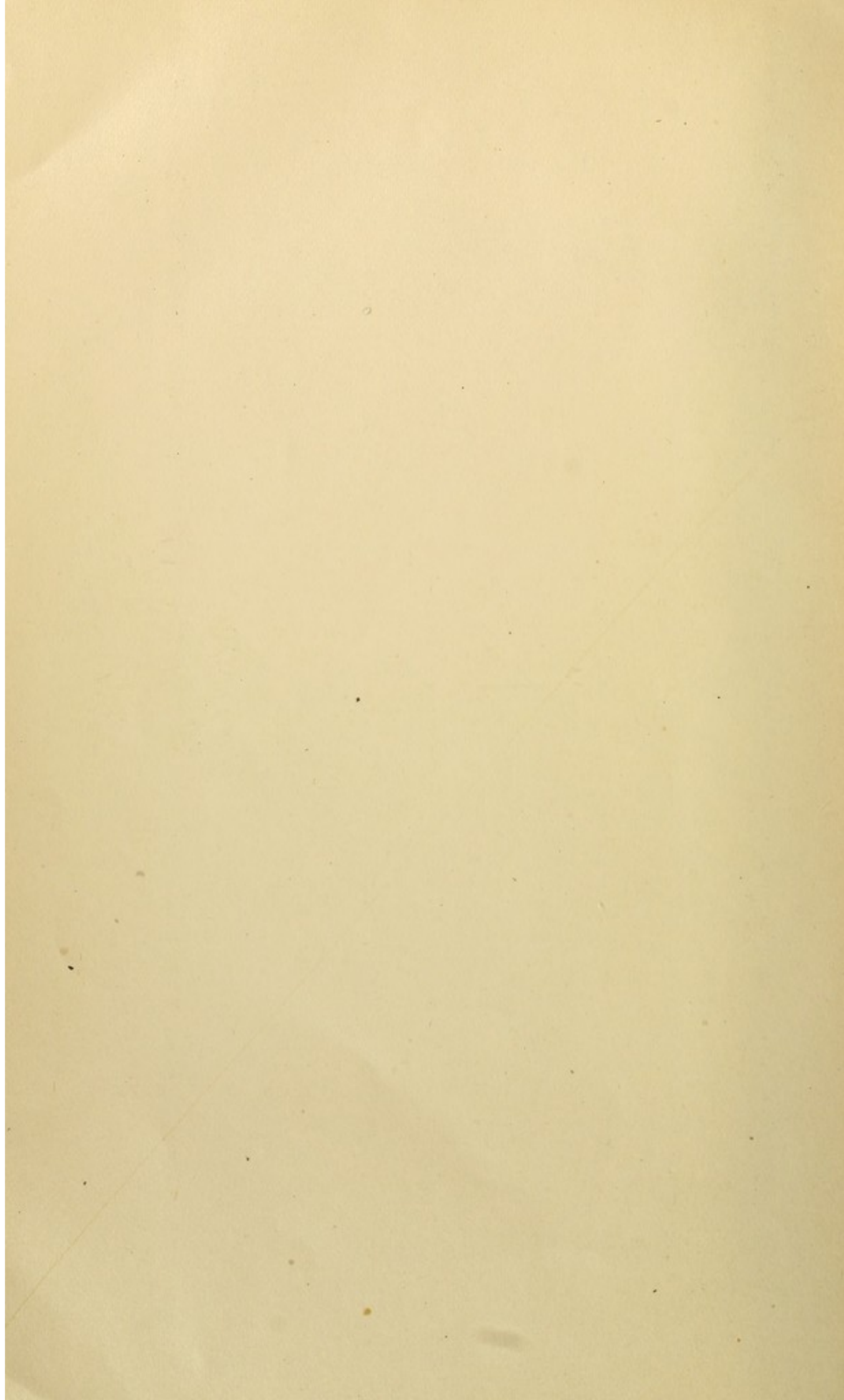
be transformed into sugar or glycogen. In alkaptonuria, however, while deamidization of alanin may occur as normally, oxidation and other changes stop at the uroleucic or homogentisic acid stage, the body cells having lost the power of splitting off or burning the benzol ring; an anomaly in intermediary metabolism without doubt due to the absence or inhibition of the specific intra-cellular enzyme which normally accomplishes this transformation.

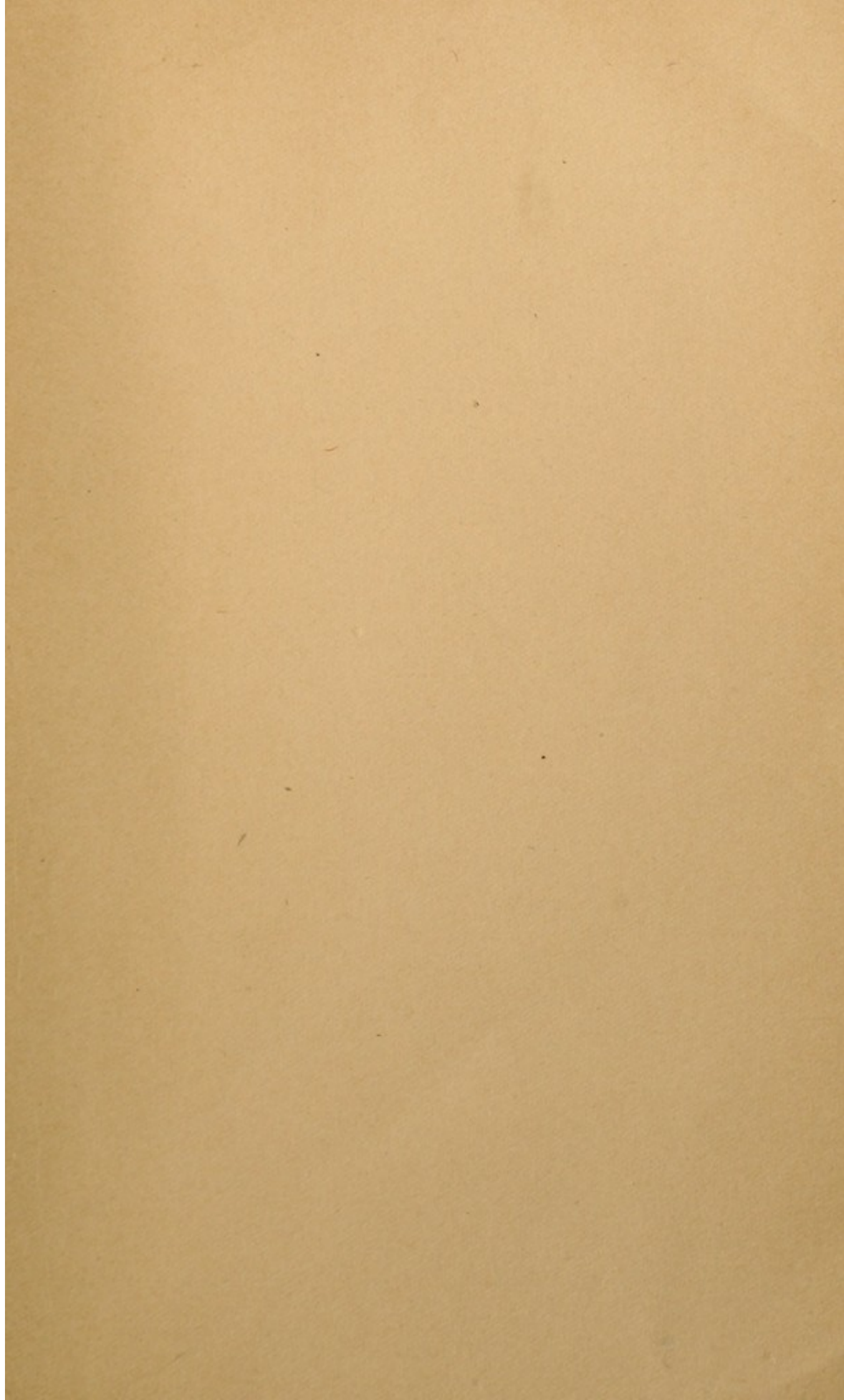
And so we see on every hand in studying the many and varied processes of intermediary metabolism, of which the foregoing are to be taken merely as illustrations, the significant part played by intra-cellular enzymes in bringing about the normal and abnormal changes characteristic of health and disease.* The processes which we have heretofore considered as due to the peculiar vital properties of the tissue cells, and which were supposed to be entirely dependent upon their morphological and functional integrity, we now see are due — certainly in large measure, if not wholly — to a great variety of enzymes which can be separated from the cell substance and which retain their activity even when free from the influence of the living protoplasm. Further, we find ample evidence that the varied processes of katabolism are the result of orderly chemical changes, of a progressive character, in which cleavage, hydration, reduction, oxidation, deamidization, etc., alternate with each other under the influence of specific enzymes, where chemical constitution and stereochemical configuration of the various molecules are determining factors in the changes produced. It is therefore evident that our understanding of intermediary metabolism will depend in large measure upon the intelligent application of the principles and methods of physiological chemistry in our study of these important and far-reaching problems.

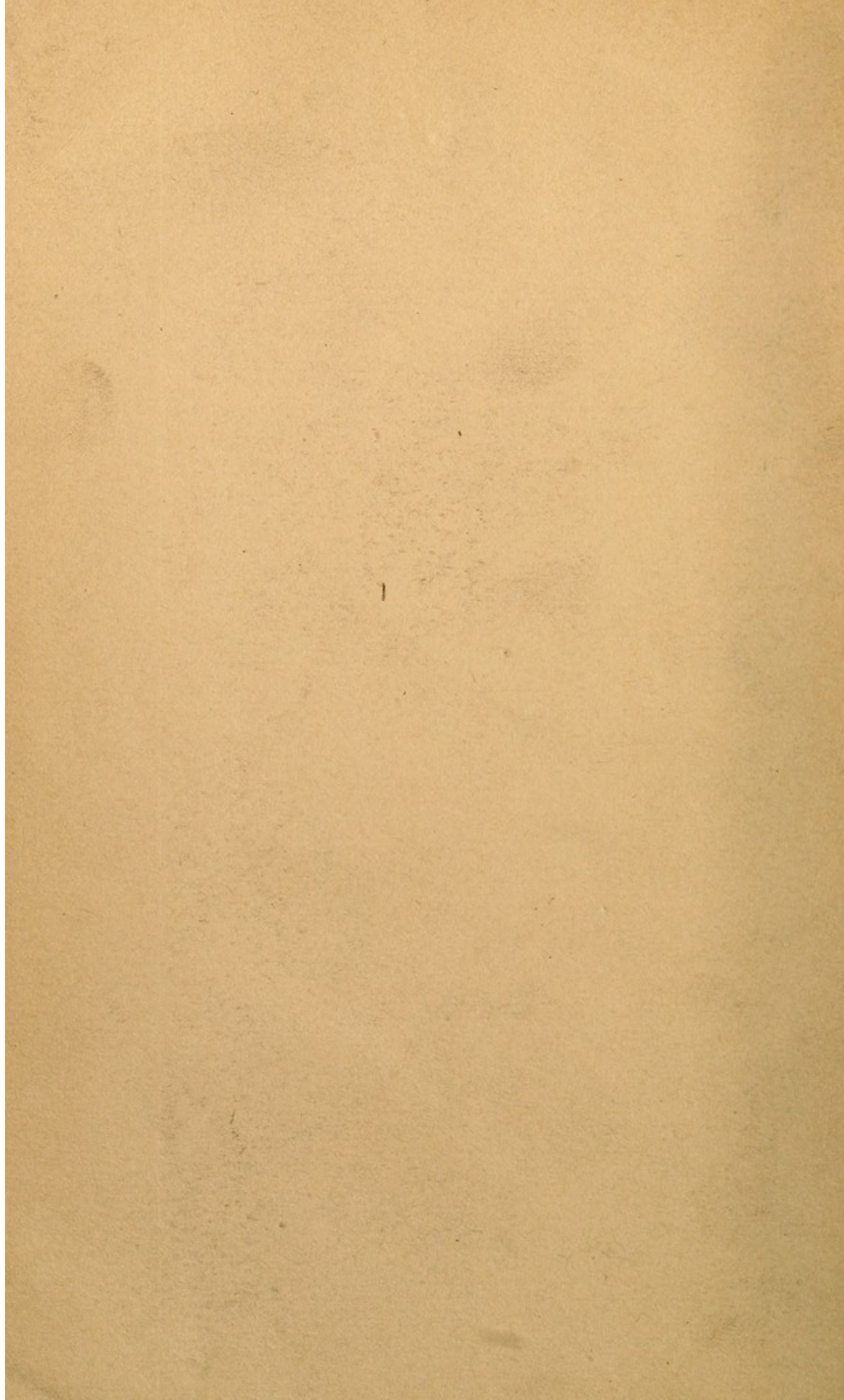
* See A. Oswald: Die Bedeutung der intracellulären Enzyme in der Pathologie. Biochemisches Centralblatt, Band 3, p. 365.

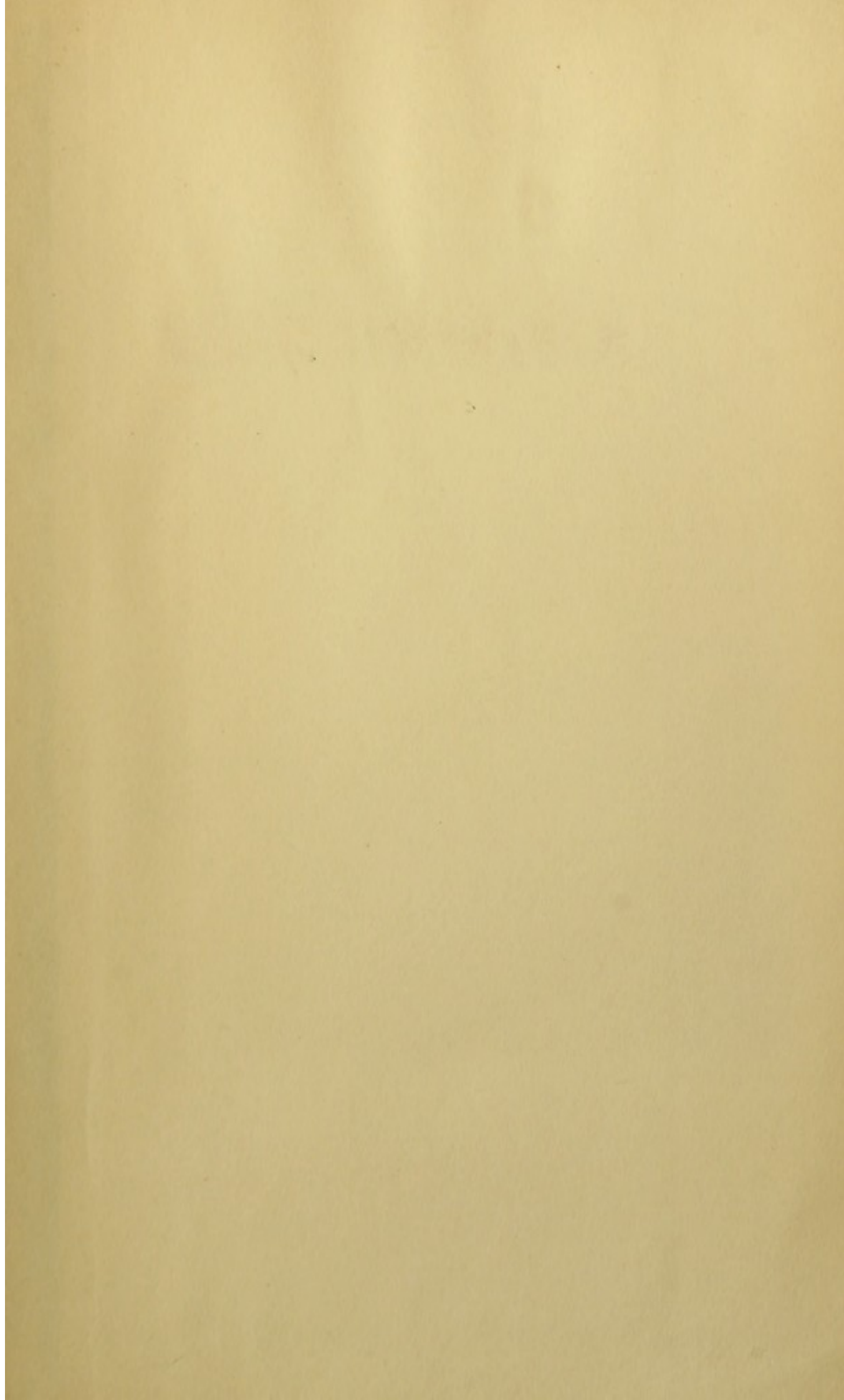












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Chittenden

Some problems of intermediary
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