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# NEW CONTRIBUTIONS IN STEROL METABOLISM

By Professor RUDOLF SCHOENHEIMER

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IT is only a relatively short time since we assumed that plants only could synthesize complex compounds whereas animals were forced to obtain these complex compounds indirectly from plants and that in modifying these complex compounds for their specific needs only slight chemical changes are necessary.

One of the most complicated substances in the animal body is cholesterol. It is a hydroaromatic secondary alcohol with 27 carbon atoms containing two combined six carbon-rings, one five carbon-ring, a side chain and one double bond.

It is, therefore, not surprising that this knowledge of the constitution of cholesterol led to the assumption that the animal body was forced to obtain this or a similar substance from plants because we could not conceive of synthetic activities of that order in animal tissues. However, cholesterol-balance studies by various authors (Thannhauser, Bürger, Beumer, Randles and Knudson) indicated that at least under certain special conditions, animals also possess the power to form cholesterol because they sometimes found a negative balance in their metabolism studies, that is, they sometimes found more sterol excreted than consumed.

These observations left unanswered the question of whether all the cholesterol present in the animal body was due to a synthesis in the animal body or whether the major part did not after all come from vegetable food. The conversion of plant sterols into cholesterol in the animal body requires that the plant sterols which differ chemically from cholesterol must be absorbable, a question which up to the present has not been investigated in detail.

The only phase of sterol absorption in the animal body known up to the present time is that of the absorption of animal cholesterol. Considerable evidence in favor of this view had been accumulated.

[1] Alpha Omega Alpha lecture delivered in Cleveland, February 27, 1931. These studies were in part aided by a grant from the Douglas Smith Foundation for Medical Research, University of Chicago.

A somewhat crude but very significant observation in this connection is the so-called experimental atherosclerosis in rabbits. These animals which are accustomed to a purely vegetable diet are very sensitive to the addition of cholesterol to their diet, in that one observes after a certain experimental dietary period morphologically visible deposits of cholesterol and cholesterol esters in various organs and especially in the aorta, a change which presents a most remarkable resemblance to the human atherosclerosis. The aorta of the rabbit is especially sensitive toward the oral administration of cholesterol), because even the daily feeding of a few milligrams if continued over a longer time leads to such deposits (Anitschow).[2]

If the above referred to theory of the simple conversion of plant sterols into the animal cholesterol were correct every rabbit should show a marked atherosclerosis, because the quantity of plant sterols present in the usual feed of rabbits is approximately ten times as high as the dose of cholesterol which calls forth an atherosclerosis. It is, however, a fact that a true spontaneous atherosclerosis with deposition of cholesterol has never been observed in rabbits in spite of the most painstaking investigations. This difference between theory and fact lead to our investigations on plant sterols.

Although practically only one sterol is found in animal tissue we, nevertheless, find a great number of different sterols in plants; these usually occur in mixtures and are separated from each other with great difficulty. Cholesterol does not even occur in traces in plants. The most commonly occurring plant sterol, which is never absent in higher plants, is sitosterol which has the same elementary formula as cholesterol, has almost identical chemical properties and which, according to Windaus,[3] is a stereo-isomer of cholesterol.

Our first experiments were carried out with sitosterol, of which larger amounts were available. We have fed enormous doses of sitosterol over long periods to rabbits and other animals, which would have suffered most severe pathological changes if cholesterol had been given instead, but the animals remained healthy and showed not even the slightest change.[4] The chemical examination of these animals showed that they contained no more cholesterol than the normal ones while those fed on cholesterol contained approximately twice the amount. The blood cholesterol concentration which is very much increased by cholesterol feeding remained normal in the sitosterol fed animals throughout the entire period. We have, furthermore, separated the total sterols from animals which were kept for almost one year on the sitosterol containing diet and have examined these sterols by Böhmer's method for sitosterol, which would detect concentrations of about 2 per cent. sitosterol. However, no sitosterol was found.[5]

Furthermore, feeding experiments on rabbits with quantitative collection and analysis of the feces showed within the experimental errors of the method that the sitosterol fed is completely excreted in the feces while the food cholesterol is similarly recovered only to the extent of about 50 per cent.[6]

All these experiments made it appear probable that the plant sterols are not absorbable just as we had assumed, but it was by no means still impossible that they may have been absorbed, then rapidly changed to cholesterol but followed by a very rapid excretion so that we would not be able to find them in the animal tissues by our method. If this were the case one would have found in part cholesterol or one of its derivatives in the feces from the plant sterol fed animals, because plant sterols do not occur in animal tissues. We have, therefore, examined the excreta of various animals when kept on a purely herbivorous diet.[7] Rabbits were kept on the usual diet of hay and beets from which a well characterized sterol mixture could be separated. From the feces the same sterol mixture could be recovered, that is, this plant sterol mixture had passed through the alimentary tract without being altered qualitatively or quantitatively.

Later more exact studies were carried out which very specifically prove that plant sterols are non-absorbable.[8] A large part of the food, especially the fats and lipins, after absorption pass through the thoracic duct. When an absorbable sterol, such as cholesterol, is fed it can be found in large amounts in the thoracic duct lymph. The examination of the thoracic duct lymph during the absorption period, is, in spite of the experimental difficulties, nevertheless the most ideal and safest method for determining the absorbability or non-absorbability of a sterol. One adds to the sterol to be tested a definite amount of the easily absorbable cholesterol and then one attempts to determine quantitatively the cholesterol as well as the other sterol content in the thoracic duct lymph. The relative proportions of the two sterols found will indicate how much more difficultly the other sterol is absorbed. This method is, of course, applicable only to those substances which can be quantitatively determined in presence of cholesterol. Un-

[2] Anitschkow, *Virch Arch.*, 249, 73, 1924.
[3] Windaus and Rahlén, *Ztschr. Physiol. Chem.*, 101, 223, 1918.
[4] Schoenheimer and Yuasa, *Ztschr. Physiol. Chem.*, 180, 5, 1929.
[5] Schoenheimer, *Ztschr. Physiol. Chem.*, 180, 16, 1929.
[6] Schoenheimer, *Ztschr. Physiol. Chem.*, 180, 24, 1929.
[7] Schoenheimer, *Ztschr. Physiol. Chem.*, 180, 36, 1929.
[8] v. Behring and Schoenheimer, *Ztschr. Physiol. Chem.*, 192, 97, 1930.

fortunately this is not true in all cases. In such cases special methods had to be devised, the discussion of which I can not go into in this brief review.[9] Our lymph studies uniformly showed that we obtained only pure cholesterol in the lymph when a mixture of cholesterol and plant sterols was fed.

From these studies one can definitely conclude that plant sterols are non-absorbable and it is, therefore, not surprising that rabbits remain normal after being fed of these substances. The results, however, also lead to the conclusion that animals which live on plants only and therefore never consume the absorbable cholesterol, are forced to synthesize their entire cholesterol necessary for their tissues.[10] Similarly since the carnivorous animals receive cholesterol directly from their food and always in the last analysis from an herbivorous animal which synthesizes its own cholesterol, we can conclude that all cholesterol is a synthetic product produced in the animal body and that a sharp biological division exists in the sterols of the plant and animal kingdoms.

However, all these experiments involved the use of chemical methods which introduce slight experimental errors. For this reason, we could not exclude the possibility that we might, nevertheless, have absorbed and deposited the minutest traces of plant sterols which were not detectable by our methods.

This is further suggested by the fact that throughout the animal organism one always finds with the cholesterol a slight trace of ergosterol which, in spite of the very small amount present, is of great biological importance, because it assumes antirachitic activity after exposure to ultra-violet light. The ergosterol found in the animal body is regarded as a plant sterol and its occurrence in the animal organsim was explained on the assumption that it is absorbed from vegetable foods and then transported to the tissues.

We finally also investigated the behavior of ergosterol, mainly because its detection with the aid of its absorption spectrum and the biological method is 1,000 to 10,000 times more sensitive than that of the other plant sterols. In such a case even a very, very slight absorption would not escape detection. The optical determination of the preparations separated by us were very kindly carried out through the courtesy of Professor Windaus in Göttingen, who also aided us in many other phases of our work. We fed rats, mice and rabbits for a long period with perfectly pure ergosterol which was entirely free from the activated form and possessed no antirachitic action. The feeding as well as the isolation of the sterols was carried out in the dark. It was found that the ergosterol fed animals yielded body sterols with no higher content of ergosterol than those from the unfed animals.

The above is in the strictest sense not final evidence of the non-absorption because we obviously determined only the possible storage of ergosterol. For that reason, the thoracic lymph was examined also.[11] Dogs were given a fatty meal to which were added one gram pure ergosterol and one gram pure cholesterol. The cholesterol preparation separated from the lymph was found to contain less than 0.02 per cent. ergosterol, a concentration which is less than that found in most cholesterol preparations. If the cholesterol and ergosterol had been equally well absorbed, the cholesterol preparation obtained from the lymph should have had about 2,000 times the observed ergosterol content. This experiment hardly permits of any other interpretation than that unirradiated ergosterol is not absorbable and that the exceedingly low concentration of ergosterol found in the thoracic duct lymph must have originated from other sources since the thoracic duct carries fluids from other sources than the digestive chyle. To be sure our experiments are not absolutely fool proof. They show, however, that ergosterol is absorbed only very slightly, so slightly that the very sensitive method used does not detect it. One can, however, not exclude the possibility that vanishingly small but biologically important amounts of ergosterol may, nevertheless, be absorbed over a long time period. The question as to whether the animal body may also be able to synthesize its own ergosterol I shall discuss later.

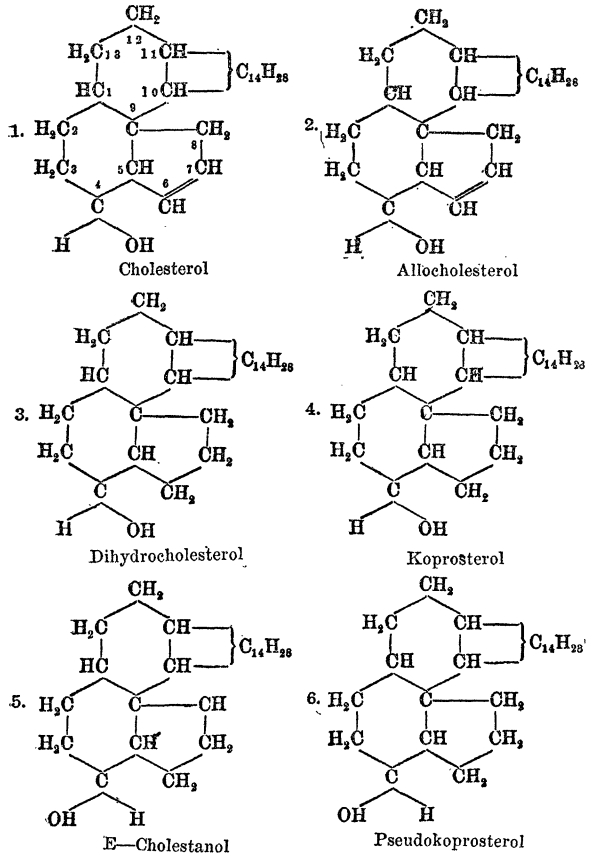
I have specifically referred to non-irradiated ergosterol, that is, not to the irradiated biologically active form which is also referred to as vitamin D. There is no doubt whatever that the irradiated form is very easily absorbed, otherwise it could not show its vitamin action and when fed in large doses even show toxic action. However, the irradiated ergosterol is a very different substance chemically from the non-irradiated form, in fact, we know that it is a stereoisomer. The fact that unirradiated ergosterol is absorbed with great difficulty or, not at all, while the irradiated form is very easily absorbed, leads to the same relationships as in the case of cholesterol and sitosterol which are also considered to be stereoisomers and where the one is easily absorbed and the other is not absorbable. In the course of our investigations we have thus opened up a field which shows that absorbability in the case of sterols is dependent upon chemical constitution and that even slight changes in the molecular structure are sufficient to

[9] Schoenheimer, v. Behring and Hummel, *Ztschr. Physiol. Chem.*, 192, 117, 1930.

[10] Schoenheimer, *Ztschr. Physiol. Chem.*, 185, 119, 1929.

[11] Schoenheimer and v. Behring, *Klin. Woch.*, 9, 1309, 1930.

render an absorbable substance completely non-absorbable. As a result, we have undertaken a systematic study involving experiments on derivatives of the easily absorbed cholesterol.[9] We studied only those substances in which only very slight changes in molecular structure are involved and in which the sterol character is still present, because we knew that even the slightest structural changes influenced absorbability.




The formulae above indicate the derivatives we have studied. In addition to cholesterol there occur in the animal body two derivatives; these occur in the feces and are sterols in which the unsaturated bond of cholesterol has been saturated by hydrogenation. These two substances, which I shall refer to again later, are koprosterol and dihydrocholesterol, two isomers which differ from each other only in a cis-trans-isomerism of carbon atom No. 1. The dihydrocholesterol is obtained directly from cholesterol by the addition of hydrogen while koprosterol is related to allocholesterol, in which the same isomerism exists with reference to carbon atom No. 1 and which was prepared only recently by Windaus. This substance is exceptionally labile in that it readily rearranges into cholesterol under the influence of heat or of dilute acids. For these reasons we must take into account in feeding experiments with allocholesterol the possibility of the acidity of the gastric contents converting a part thereof into cholesterol and thus rendering it absorbable. The comparative absorption studies on cholesterol and allocholesterol showed that the latter is absorbed much more difficultly than cholesterol (about one half to one third as much). It is, however, by no means impossible that the absorbed allocholesterol actually was first converted into cholesterol in the stomach.

The other derivatives of cholesterol, especially those which also occur in the organism and which can be separated in traces from cholesterol also were non-absorbable even in traces.

These observations then led to the conclusion that the organism behaves in an exceptionally specific manner in the absorption of the sterols. Even isomerism suffices to alter the absorbability and the saturation of the unsaturated bond changes the easily absorbable body to a completely unabsorbable form.

An absorption, which is so specifically dependent upon molecular structure, has previously not, to my knowledge, been systematically investigated. The observations led to an approach at interpreting the difficult or non-absorption of sitosterol and unirradiated ergosterol and the ease with which cholesterol and irradiated ergosterol pass through the intestinal wall.

We have also extended our investigations to the consideration of the waste products or, better stated, the products of intermediary metabolism of cholesterol, a field regarding which almost nothing was known up to the present. I pointed out that cholesterol does not appear as such in the feces but as the two saturated forms, koprosterol and dihydrocholesterol. The former of these occurs in far greater amounts.

The general view held up to the present as to the formation of these bodies was that the cholesterol from food, bile or digestive juices enters the intestines and is there converted into these products by the action of intestinal bacteria. We have here under consideration a reaction involving hydrogenation, that is, a reduction, a reaction long known to be easily produced by intestinal bacteria, as for instance the conversion of the unsaturated bilirubin into the saturated urobilin-like body. Although the conversion of bilirubin into urobilin has been successfully carried out *in vitro* by putrefactive organisms, all attempts by others as well as by us[12] with the *in vitro* conversion by bacteria of cholesterol into koprosterol and dihydrocholesterol have led to negative results.

In order to clarify the origin of these saturation sterols we undertook another method of attack. We, contrary to the earlier investigators, postulated that the saturated sterols did not originate in the intestines

[12] Schoenheimer, v. Behring, Hummel and Schindel, *Ztschr. Physiol. Chem.*, 192, 73, 1930.

but that they represent intermediate products of metabolism, that is, that they are formed in the tissues themselves and are excreted into the intestinal contents. For these studies it was necessary to develop a method which is sufficiently accurate to determine traces of these saturated sterols when mixed with cholesterol.[13] This method is based upon the observation that all sterols form difficultly soluble addition compounds with digitonin but that the bromine derivatives of the saturated sterols, that is the cholesterol dibromide which is easily and quantitatively obtained by treating a solution of the sterol with free bromine, are no longer precipitable by digitonin. Obviously one simply needs to treat the sterol mixture carefully with bromine and then the addition of digitonin precipitates only the saturated sterols originally present because the absence of the double bond prevents the formation of bromide derivatives of the saturated sterols.

With this method we could, to our surprise, establish the fact that all cholesterol preparations, immaterial from what animal or human organs they are obtained, contain such saturated sterols, usually small in amount.[14] In most cases not more than two to three per cent. is present, but none of the sterol preparations were found free from these substances. The fact that these substances are present in such small amounts explains why they were not detected before.

We succeeded in separating these saturated sterols in pure form by working with large quantities of the various cholesterol preparations.[15] We expected, of course, that we would find a mixture of a composition very similar to that found in feces, that is, a mixture of koprosterol and dihydrocholesterol with the former in great excess. Contrary to these expectations we found only pure dihydrocholesterol and no trace of koprosterol could be detected.

The same observations were made on plant sterols. All available plant sterol preparations contained in addition to the main component, the unsaturated sterols, varying amounts of saturated sterols. It appears, therefore, that the occurrence of sterols as mixtures of the saturated and unsaturated kinds is a common biological observation.

Naturally our first studies were on animals. Where do the saturated sterols originate? I have already stated that these substances which always occur in large amounts in the intestinal contents are non-absorbable and are not, as we assumed formed in the intestines by the action of bacteria on cholesterol. There is, therefore, no other explanation left for us than to state that the dihydrocholesterol is formed in the tissues by the hydrogenation of the cholesterol and that it is to be considered a product of cholesterol metabolism.

The real question next was by what path does dihydrocholesterol thus formed in the body pass into the intestines? Formerly it was assumed that the main part of the cholesterol from the body passed to the intestines by solution in the bile. Therefore, it was necessary to assume that very likely gall stone cholesterol would also contain very large amounts of dihydrocholesterol. We found, however, only very small amounts. The calculations then indicated that still another source must exist. Sperry[16] some time ago showed that the intestinal wall is permeable to lipoids and that we have excreted with them some cholesterol. We, therefore, suspected that the sterol thus excreted probably was not cholesterol at all, but dihydrocholesterol instead.

In order to prove this we found it necessary to carry through the isolation of sterols from large amounts of sterile intestinal secretion, an undertaking which presented tremendous experimental difficulties. The following procedure was found satisfactory.[17] We sectioned the small intestine of a dog above the large intestine and sewed the lower end of the small intestine to an opening in the abdominal wall. The isolated end of the intestine which was still united to the anus was rinsed with many liters of water until the washings were obtained perfectly clear, then the upper end of this intestinal section was lighted. The blind intestinal section thus consisted of the large intestine and a small part of the small intestine and still connected to the anus. After two days this intestinal preparation was rinsed daily from the anus with warm water. After about eight days of such treatment the rinsings from this intestinal preparation were found almost sterile. If the anus was now closed by operative procedure the antibacterial action of the intestine was sufficient to destroy the remaining microorganisms.

After one to two months the animal was sacrificed and the contents of the intestinal preparation recovered. The content was very interesting. In most cases it was completely sterile. Usually we obtained per day 60 to 70 grams of a light yellow, wax-like mass, the surface of which presented in relief the character of the mucous membrane of the intestine. We have no doubt that we have here the normal dried intestinal secretion which normally would be mixed with the feces and therefore would escape detection. It has only a small water content and all reabsorbable substances, including cholesterol, must have been reabsorbed, that is, we have here those substances which

[13] Schoenheimer, *Ztschr. Physiol. Chem.*, 192, 77, 1930.
[14] Schoenheimer, v. Behring and Hummel, *Ztschr. Physiol. Chem.*, 192, 93, 1930.
[15] Schoenheimer, *Ztschr. Physiol. Chem.*, 192, 86, 1930.
[16] Sperry, *Jl. Biol. Chem.*, 82, 560, 1929; *ibid.*, 85, 455, 1930.
[17] Schoenheimer and v. Behring, *Ztschr. Physiol. Chem.*, 192, 102, 1930.

are secreted but not reabsorbed. From these masses of material we could separate very significant amounts of dihydrocholesterol, but koprosterol was not found.

As a result of these observations we had proved that the dihydrocholesterol after its formation in the tissues actually is secreted through the intestinal wall. This clarifies the metabolism of dihydrocholesterol but not of koprosterol. Since koprosterol was not found in the tissues but always in larger amounts in the feces, we must still assume that it is a product of the bacterial action in the intestines even though we have not been able to reproduce the same *in vitro*. We must consider that up to the present it has not been possible to cultivate all of the intestinal bacteria and it is very probable that any one of the difficultly cultivatable anaerobes may be able to bring about this change. It is hoped that the near future may throw light on this question.

To us the most important question now appeared to be, what is the significance of this intermediary hydrogenation which leads to the formation of dihydrocholesterol in the animal? The question may also be formulated to read: What is the source of the hydrogen which leads to the hydrogenation of the double bond in cholesterol? In view of this process existing in both animals and plants we must conclude that we have under consideration a general biological process in which the cholesterol functions as hydrogen acceptor. According to the Wieland dehydrogenation theory, it was necessary to assume that this hydrogen originated from other organic substances which yield hydrogen and thus became unsaturated. This hydrogen yielding substance was expected to be found among the other lipoids associated with which cholesterol occurs in nature.

Since the amount of dihydrocholesterol formed daily is very small, a working hypothesis was advanced that the formation of dihydrocholesterol is associated with the formation of fat soluble, highly unsaturated, but biologically very active substance. Such a substance, for example, would be ergosterol which possesses a structure very similar to cholesterol, but which has three double bonds instead of one as in cholesterol. I stated that ergosterol is very difficultly, if at all, absorbable and since it is present in all animal tissues it led to the view that animals as well as plants possess the power of forming the ergosterol. If this is the case, then it is also possible that we might have formed simultaneously from cholesterol both dihydrocholesterol and an ergosterol-like body.

Recently Koch, Koch and Kraus-Ragins,[18] carried out a very illuminating experiment. When cholesterol, freed from the last traces of ergosterol, was heated at a high temperature without access to oxygen, a new substance was obtained which, after irradiation by ultra-violet light, possesses antirachitic activity.

We have modified these experiments for our purposes and have worked with cholesterol preparations which were not only free from the last traces of ergosterol but also of dihydrocholesterol.[19] When these preparations were heated to high temperatures in a high vacuum with a complete exclusion of oxygen there was obtained a small amount of a saturated sterol which very likely is dihydrocholesterol. At the present time we have only a very small amount of this difficultly obtainable substance, so that we have not been able to identify it accurately. The formation of this saturated sterol and of the antirachitic body can, however, not be explained in any other way than to suppose that a small part of the cholesterol has been converted by dehydrogenation into an ergosterol-like body with the probable introduction of two new double bonds and that at the same time there is formed in another part of the cholesterol a completely saturated sterol due to the liberated hydrogen.

We have not always succeeded in carrying out this reaction because it appears that a catalytic agent may be involved. This reaction makes it appear very probable that the animal body may also be able to carry out a similar process and that the dihydrocholesterol found by us is in fact associated with the formation of the ergosterol. This interpretation must, however, be confirmed by further experimentation.

In this lecture I have referred only to the most obvious results of our work. On account of lack of time I have unfortunately not been able to discuss the methods which are so very important in all experimental work and serve as a basis for a critical evaluation.

# NATIONAL PARKS IN AFRICA

## THE EXTENSION OF WILD-LIFE CONSERVATION

By MARY L. JOBE AKELEY, A.M., Litt.D.

SECRETARY OF THE AMERICAN COMMITTEE FOR THE PARC NATIONAL ALBERT

THE first national park created in the great continent of Africa is the Parc National Albert, established by royal decree of Albert, king of the Belgians, in 1925. Here, in the Kivu District of the Belgian Congo, are found the rare mountain gorilla (*gorilla gorilla berengei*), to-day of increasing scientific importance. Living side by side with the gorilla on the

[18] Koch, Koch and Kraus-Ragins, *Jl. Biol. Chem.*, 85, 102, 1929.

[19] Schoenheimer, *Naturwissenschaften*, 18, 881, 1930.