thought, however, that this point might be worth investigating. Perhaps, then, some definite regions of origin might be discovered.

DR. NORMAN BRIDGE, Los Angeles, Cal., said that he failed to hear mentioned the one medicament of great value in chlorosis. This drug is pre-eminently valuable in chlorosis as a therapeutic measure capable of increasing the hemoglobin of the blood. It has been shown, and clearly enough, he believes, that iron in many of these cases is of little value. The one drug he had in mind was calomel in tonic doses—not cathartic doses—that is, from 1/50 to 1/30 of a grain, given three times a day, but not oftener. How it works or operates he does not know or pretend to know. But that it has a power vastly greater than the iron preparations in securing good blood and increasing the iron in the blood, there was no question in his mind. It should be given at least a month continuously, and there is, of course, no objection to giving at the same time iron or any better tonic—or all together.

DR. C. H. BUNTING, Charlottesville, Va., referred to the multiplicity of the causes of pernicious anemia. Dr. Cabot has divided this type into pernicious anemia and the anemia due to parasites. Experimental work has shown that one may expect to find a multiplicity of causes, because various toxins can be used to produce the same picture in animals. In human pathology, then, one may have a variety of causes producing the condition in the blood which is described as the picture of pernicious anemia. He believes that the great majority of the cases, however, which are recognized as cases of pernicious anemia are due to practically the same cause; this is true, although clinically and pathologically one can not separate the cases which are due to a parasite from those which are due to unknown causes. As to the treatment, there is one suggestion to be made from the pathologic side: Rest, fresh air and diet are very important in enabling the bone marrow to produce and reproduce new red cells. But the essential thing must be the removal of the cause. In the *Bothriocephalus latus* anemias we have a good example of what results when the cause is removed, recovery. Consequently, he believes that we have one certain indication in every case of pernicious anemia, and that is the careful treatment of the gastrointestinal tract.

DR. BAILEY K. ASHFORD, Washington, D. C., said that he approved of Dr. Hutchinson's remarks regarding the influence of iron. Iron is of value in certain cases of uncinariasis in which the recovery is slow; in such cases iron does well. But ordinarily iron is not necessary. As to the possibility of uncinariasis being produced by a toxin, we have not yet been able to demonstrate any such toxins, but there are certain facts which might make this probable in uncinariasis. He wished to call attention in this connection to the hyaline degeneration of the arteries of the Malpighian corpuscles of the spleen.

DR. RICHARD C. CABOT, Boston, said that Dr. Bunting's classification of anemias into primary and secondary is simply a question of convenience; there is the same clinical picture and pathologic anatomy in the anemia from *Bothriocephalus latus* as in pernicious anemia. It is merely convenience that makes us call one primary and the other secondary. Dr. Cabot said that Dr. Ashford's work in Porto Rico is really a campaign against a disease of the whole people, for a considerable majority of the population of Porto Rico have uncinariasis. Now through the energy and honest effort of Dr. Ashford the island is likely to be freed of the disease. Dr. Cabot said that he never hears or speaks of this disease but that he feels the greatest debt of gratitude to Dr. Ashford for what he has done toward saving the population of this island from inefficiency and from disease. In regard to the existence of pernicious anemia in the rural districts Dr. Cabot said that out of 337 cases he has seen, more than two-thirds come from the rural districts; whether a coincidence or not, this certainly is a striking fact. Pernicious anemia will be found to be a common disease in every part of the country if it is looked for. Pernicious anemia is ten times more common than myxedema and five times more common than leukemia. Dr. Cabot has tried saline injections, as he has tried many other treatments for pernicious anemia. Out of the 337 pa-

tients with pernicious anemia three are alive, and he does not believe they got well by reason of what he did for them. This fact made him rather slow in accepting anything in the way of therapeutic measures, whether intestinal flushings, or arsenic, or rest, fresh air, which he always gives, or anything else. He has never been able to make up his mind that arsenic does any good.

DR. S. J. MELTZER, New York, said that it is not fair to bring up here the fatal case in Philadelphia as an argument against the value of direct transfusion as a method. The case may prove, if it proves anything, that in certain pathologic states transfusion is inadvisable. Crile, however, introduced the method of direct transfusion primarily for anemias caused by hemorrhages of traumatic origin. In these cases the direct transfusion apparently renders excellent service. The method, therefore, marks a distinct progress in the treatment of anemia, and that is all Dr. Meltzer claimed for it. The celebrated clinician, Niemeyer, is quoted as saying that his success was due to the use of iron in chlorosis. He gave Bland's pills in large quantities. Dr. Meltzer would advise other physicians to do the same, the opinion of some physiologic chemists and hematologists notwithstanding. The history of the pharmacology of iron for the last twenty-five years teaches that, in the practice of medicine, the clear-sighted clinician is very frequently a safer guide for the practitioner than physiologic chemists, some of whom venture to offer positive opinions in fields in which they possess neither theoretical nor practical personal experience.

AN INVESTIGATION OF THE NATURE OF PROTEID-SOAP COMPOUNDS AND OF THE STAINING OF PURE FATS AND LIPOIDS BY SCHARLACH R AND SUDAN III.*

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These studies were undertaken for the purpose of further investigating certain reactions on which Klotz¹ bases in large part his interpretation of pathologic calcification and which, as his interpretation is not in accord with that reached by Wells² and independently by me,³ are matters of considerable importance in the elucidation of this interesting problem.

The observations in question are those concerning the value of the so-called proteid-soap compounds and of the staining of fats and lipoids by Sudan III and Scharlach R.

PROTEID-SOAP COMPOUNDS.

Klotz, as the result of a series of microchemical examinations of various kinds of calcified tissues, concluded that the deposition of lime in tissues undergoing calcareous degeneration is dependent on the formation of calcium soaps as an intermediate step. He further states that an essential factor in the production of these soaps is the initial combination of a soluble soap and an albuminous substance. He does not assume, however, that this is the essential process in all varieties of pathologic calcification, but states that in exceptional cases there may be two other possibilities: First, "that under certain conditions the calcium salts act directly on the neutral fats present in the degenerating cells,"

* From the Department of Physiological Chemistry, Bender Laboratory, Albany, N. Y. This investigation was pursued under a grant made by the Committee on Scientific Research of the American Medical Association.

1. Studies on Calcareous Degeneration, I. The Process of Pathological Calcification, Jour. Exp. Med., 1905, vii, 633.

2. Pathologic Calcification, Jour. Med. Research, 1906, xiv, 491.

3. Chemistry of Atheroma and Calcification (Aorta), Jour. Med. Research, 1906, xv, 355.

or, second, "that the neutral fats are first broken down into fatty acids and that these react with the calcium salts to form the soaps."

As he was convinced that the combination of soaps with proteid is a necessary intermediate product in certain forms of calcareous degeneration, he attempted to reproduce the reaction in the test tube. To a weak solution of a soap (sodium stearate, oleate or palmitate), a dilute solution of egg albumen was added. In a half hour or longer, at room temperature, a white flocculent precipitate appeared. This he considered to be a combination of the soap and the egg albumen. When this product was treated with Sudan III the same pinkish color, visible in the "soap" granules in tissues undergoing calcification, was seen to be present. The amount of precipitate, he states, is further increased by the passage of carbon dioxid (CO_2) through the solution. Acid calcium phosphate, which does not affect simple solutions of egg albumen, when added to a mixture of egg albumen and soap causes a rapid and dense precipitation.

As microchemical reactions in tissues are notoriously unreliable and controlled only with great difficulty, these reactions in the test tube necessarily formed the basis of Klotz's theory and their explanation is, therefore, of the greatest importance.

The first step in the present investigation was to determine the nature of this precipitate, that is, whether a real combination of proteid and soap does occur, and if not, to ascertain the actual composition of the precipitate. These experiments were carried out with egg albumen and the blood serum of horses. The soluble soaps used were sodium stearate and sodium oleate. In repeating the experiments of Klotz an identical reaction was obtained, that is, on the addition of the soap solutions to the egg albumen or horse serum a flocculent precipitate appeared which gradually increased in amount and reached its maximum at the end of twenty-four hours. In order to determine whether or not this was the result of a true combination of proteid and soap or merely a precipitation of some substance in the mixture, the following experiment was performed: Two lots of 100 c.c. each of horse serum were taken; to one, 100 c.c. of distilled water were added, and to the other 100 c.c. of freshly-prepared 5 per cent. sodium oleate solution. At the end of twenty-four hours a finely granular precipitate was visible in the serum-water mixture, while in the serum-soap solution a copious flocculent precipitate appeared. These were filtered and yielded clear straw-colored filtrates. In order to determine whether or not any combination of proteid had taken place in the soap-proteid mixture, duplicate total nitrogen determinations were made, by the Kjeldahl method, on aliquot parts (5 c.c.) of each of the filtrates. The results were as follows:

TOTAL NITROGEN.	
SERUM AND WATER MIXTURE.	SERUM AND SOAP MIXTURE.
N	N
20.15 c.c. of — acid.	20.15 c.c. of — acid.
10	10

From these figures it is easily seen that the nitrogen (proteid) contents of both filtrates was the same and that no more nitrogen was thrown out of solution by the soap than by the water alone. This finding is of considerable importance, for if a true combination of proteid and soap had taken place the filtrate obtained after the removal of the precipitate would have contained less nitrogen (an indication of soluble proteid) than the fil-

trate from the serum-water mixture, where no combination was suspected. As this did not occur, the conclusion is inevitable that the precipitate is not a proteid-soap compound, but must consist of a mixture of other substances thrown out of solution.

In order to throw more light on this reaction, experiments were made to determine the influence of the colloids (globulins, albumins) and salts of the serum and egg albumen in the precipitation of the soaps.

GLOBULINS.

The fact that Klotz emphasized the increased precipitation after the passing of carbon dioxid through the fluid suggested the possibility of the globulins of the serum being the main constituent of this precipitated material. It is a well-known fact that globulins are precipitable from neutral solution on addition of carbon dioxid gas. Globulins, therefore, were prepared from normal horse serum in the usual way (ammonium sulphate method with dialysis). The globulin thus obtained was dissolved in a 10 per cent. solution of sodium chlorid and a small quantity of it added to a weak solution of sodium stearate. Immediately a heavy flocculent precipitate appeared. As a control, however, a 10 per cent. solution of sodium chlorid was added to a solution of the soap, and an apparently identical precipitation occurred.

It was evident, therefore, that the material precipitated in the first instance was not the globulin but the soap. This reaction is analogous to the salting out of soaps in their commercial manufacture. In an attempt to exclude this factor the globulin was put in solution with the smallest possible quantity of salt, but in every case control experiments using solutions of the same strength of sodium chlorid showed an equal and similar precipitation. The only part then which the globulin can play in this precipitation lies in the reaction noted above in which the globulin of the serum or of the egg albumen can be precipitated by the addition of water alone.

INORGANIC SALTS OF THE BLOOD SERUM AND EGG ALBUMEN.

To a weak solution of sodium stearate, an equal quantity of physiologic sodium chlorid solution was added. As control, an equal quantity of distilled water was added to another portion of the sodium stearate solution. At the end of twenty-four hours a precipitate was visible in both tubes, but was more marked, however, in the sodium chlorid solution. In the sodium stearate and water mixture the precipitate was finely granular and, to a certain extent, diffuse, while in the soap-sodium chlorid mixture it was more flocculent and the supernatant fluid slightly translucent.

As a further control, a solution of the normal salts of the serum were obtained as follows: One hundred c.c. of horse serum were added to 400 c.c. of distilled water. The mixture was then boiled and the proteid coagulated by the careful addition of acetic acid. The filtrate, clear and colorless, was then evaporated to dryness to remove the excess of acetic acid and by the addition of distilled water was brought up to the original volume of the serum. A similar solution of salts of egg albumen was obtained after removal of the coagulable proteids. To 10 c.c. of each of the proteid-free solutions 10 c.c. of sodium stearate solution were added. At the end of twenty-four hours a precipitate was visible which, although much finer in appearance, approxi-

mately corresponded to the precipitate formed by the addition of the physiologic sodium chlorid solution to the sodium stearate. As the result of these tests it may be stated that while the inorganic salt content of egg albumen or of serum does not probably cause the whole of the voluminous precipitate obtained on addition of soap to the proteid, it is a factor in the production of a small amount of it.

In this connection attention must be called to the calcium content of the egg albumen (1.7 to 2.9 per cent. of the ash) and blood serum (.015 per cent. of the serum). In both of the reactions with these fluids and the soaps this calcium is undoubtedly a factor in precipitating this latter as calcium compounds.

ALBUMINS.

The effect of salt-free albumins was then investigated. These were prepared by dialyzing egg albumen and horse serum for about ten days or until tests for chlorids were negative. In each instance the precipitated globulins were filtered off; in the case of the serum a small amount of water soluble globulin probably still remained in solution. Comparing solutions of albumins so prepared plus stearate soaps with the control solutions of egg albumin and serum albumin respectively in water, at the end of twenty-four hours no difference was visible. In all the tubes a very small amount of a very finely granular precipitate occurred.

These reactions render it probable that the albumins do not enter into the composition of the precipitate which is formed when the soaps react with the egg albumen. In order, however, to exclude the possible factor of the albumin inducing a "colloidal precipitation" experiments were performed with other colloid substances, for instance, gelatin and starch. With gelatin a marked precipitation was apparent with the soaps, but as the admixture of calcium salts with this substance could not be eliminated, solutions of other colloids like starch were tried.

A rather fluid starch paste was prepared. Some of this was added to equal amounts of egg albumen and blood serum. In both cases a somewhat gelatinous precipitate occurred which was shown to consist of starch. Solutions of starch added to the soap solutions caused a precipitate which was analogous to that produced by water alone. Apparently, therefore, colloids may precipitate starch from solutions as well as soaps, but the reverse does not occur.

Thus far only a small amount of the precipitate has been accounted for. We have shown that the inorganic salts of the serum are responsible to a slight extent in the precipitation of the soaps; the albumins on account of their colloidal nature, possibly also in the same degree, while the globulins enter only slightly into the composition of the precipitate as a result of the dilution of the mixture by the solutions added.

FATTY ACIDS.

The changes occurring in soap solutions suggested the possibility of fatty acids playing a part in the precipitate. For example, a weak solution of sodium stearate or oleate freshly prepared and filtered is translucent and free from any precipitate. When tested with litmus it is alkaline in reaction. If it is allowed to stand for twenty-four hours a granular precipitate in the case of the stearate, gelatinous in the case of oleate, gradually appears.

If the precipitates are filtered off and the filtrates al-

lowed to stand for twenty-four hours a similar precipitate reappears. This precipitate is insoluble in water, but dissolves readily in ether and chloroform. Such a chloroform solution reacts acid to litmus and contains fatty acid. These facts just outlined can be easily explained. Part of the soap on passing into solution undergoes dissociation and hydrolysis with the production of sodium hydroxid (NaOH) and free fatty acid. The mixture, therefore, becomes alkaline and the fatty acid gradually precipitates. This passes into solution in the chloroform.

With these changes in mind, the proteid-soap compounds were examined as follows: A quantity of the precipitate obtained on the addition of a sodium oleate solution to egg albumen in suspension was now shaken up with the neutral chloroform and found to a great extent to be soluble. Tested with litmus, the chloroform reacted acid to litmus and the neutral point was only reached after the addition of a definite amount of a decinormal solution of sodium hydroxid. This observation, therefore, demonstrates conclusively that the major portion of the precipitate which Klotz considers to be a proteid-soap compound is composed of fatty acids.

Fatty acids may also be thrown out of solution on the addition of weak acids. If carbon dioxide (CO₂), which in aqueous solutions is a weak acid, be passed through a solution of soap, a flocculent precipitate gradually separates out, which when taken up in chloroform and tested, reacts acid to litmus. In the formation of the hypothetical proteid-soap compound Klotz emphasized this increased precipitation on the addition of carbon dioxide. He further states that the precipitation is increased by the addition of a solution of acid calcium phosphate. This reaction is, of course, due in part to the precipitation of the fatty acids by the acid solution and also to the formation of insoluble calcium soaps by the addition of a soluble calcium salt.

To summarize briefly: The so-called proteid-soap compound does not exist. The material may be due in small part to a precipitation of the soaps by the inorganic salts of the serum or egg albumen and in small part to globulins thrown out of solution. An equally small part is caused by a precipitation of the soaps or fatty acids by the colloid substances in the serum and egg albumen. The main portion of the precipitate, however, consists of fatty acids obtained from a hydrolysis taking place in the soap solutions.

THE STAINING OF FATTY COMPOUNDS WITH SUDAN III AND SCHARLACH R.

Klotz states that the soaps are ready solvents for Sudan III. He attempts to demonstrate this by making a concentrated aqueous solution of the soap and adding powdered Sudan III. He states that the clear deep red solution which results can be filtered, diluted and, when evaporated to dryness, yields pinkish yellow areas which are distinguishable from the golden-red staining fat. He cites the statement of Fischler, who asserts that Sudan III is soluble in soap solutions, but who further states, however, that the soap when stained with Sudan III rapidly loses its color when treated with 70 per cent. alcohol, the color being transferred to the alcohol. With this latter statement Klotz fails to concur wholly. He admits that this is true in regard to soap solutions in the test tubes, but states that soaps in the tissue are less soluble in weak alcohols. The retention of the stain there, he thinks, is due to the combination of the soaps and proteids already discussed. These obser-

variations, in view of our experience with the proteid-soap compounds, demand re-investigation.

Scharlach R and Sudan III are soluble in alcohol, chloroform, ether, toluol, acetone, etc., and in certain fats and lipoids. When added to water no color is imparted to the fluid even at the end of several days. It is generally accepted that the stains are insoluble in water.

SOLUBLE SOAPS.

If to a fairly concentrated solution of sodium oleate which has been freshly made and filtered a small quantity of Sudan III is added, after "thorough mixing," no color is immediately imparted to the fluid. Gradually, however, the solution assumes a pinkish color which, after standing for a while, becomes much deeper. At the end of twenty-four hours the solution is colored a deep red. If some of this solution is shaken with ether or chloroform the color immediately leaves the aqueous solution and is transferred to the chloroform and ether. When tested with litmus the solution reacts strongly acid, and if evaporated to dryness, reddish yellow granules of fatty acid are visible under the microscope.

The colorless condition of the soap solutions immediately after the addition of the Sudan III and the gradual reddening of the mixture must be attributed to the same reaction previously described where the soaps slowly undergo hydrolysis and the fatty acids formed thereby gradually assume the stain which eventually colors the solution a deep red. In other words, the deep orange red color is not due to the soaps which have taken up the stains, but rather to the fatty acids formed by hydrolysis and temporarily held in solution by the soaps.

CALCIUM SOAPS.

To a quantity of the original reddened aqueous solution of the soluble soap a quantity of absolute alcohol was added sufficient to raise it to a 70 per cent. alcohol solution. When this alcoholic solution was treated with a soluble calcium salt, a pinkish white precipitate appeared at the end of twenty-four hours, the supernatant fluid remaining deep orange red. The precipitated substance was filtered off; when dried it appears white and very fluffy in consistence. It is insoluble in water, but dissolves in chloroform when a few drops of a mineral acid are added. The precipitate is, therefore, calcium soap which does not take the stain with Sudan III.

In order to prove this point more decisively attempts to stain calcium soaps were made in another manner. Calcium soaps were prepared by the precipitation of the soluble soaps—sodium stearate and oleate—with a soluble salt of calcium. The collected precipitate was thoroughly washed with water to remove soluble salts and afterward with alcohol to remove traces of fatty acids. In the case of the stearate a finely granular opaque white salt was obtained, while with the oleate compound a rather gelatinous material resulted.

A relatively large quantity of the insoluble calcium stearate was suspended in an absolute alcohol solution of Sudan III. As has been previously shown, calcium soaps are rather insoluble in cold absolute alcohol. The suspended material, now filtered off, appears pinkish white. Washed with absolute alcohol, the pinkish color disappears and is imparted to the wash alcohol. The insoluble soap remains opaque white. The pinkish color of the soaps treated with Sudan III is not due to real staining, but is produced by a mere mechanical mixture of the dissolved stain and the insoluble compounds. At-

tempts which were made to stain these soaps by methods outlined below were also fruitless.

NEUTRAL FATS AND LIPOIDS.

It was deemed extremely important to determine if Scharlach R and Sudan III could be used as differential stains for the various fats and lipoids. Several methods of demonstrating this were tried. For example, the examination of frozen sections of colloids (gelatin, celloidin) and of various fresh and dehydrated animal tissues impregnated with solutions of the fat and lipoids to be examined. These methods were abandoned in favor of the following, which seemed to be more applicable to the problem:

To solutions of fats and lipoids Scharlach R and Sudan III respectively were added. The resultant clear solution was thrown into water and the fats precipitated out of their original solution. This method we considered relatively successful because of the ease with which the stained fat can be separated from the solvent. While the fats are fairly soluble in alcohol and ether, they dissolve much more readily in chloroform, which is also an excellent solvent for Sudan III and Scharlach R. Chloroform also will not mix with water, that is, it is insoluble in that liquid.

If, therefore, a chloroform-Sudan III solution of a fat is added to water the fat will precipitate out, so will the chloroform, although both may be colored red, the fat rises to the surface of the fluid while the chloroform settles to the bottom, allowing an easy differentiation. The chloroform, however, must be removed by evaporation with heat before one may say definitely that the red globules are the fatty compound and not the chloroform. In this method a portion of stain remains adherent to the floating fat, while most of the insoluble stain is suspended through the fluid. It is necessary, therefore, that this fat be not filtered, but skimmed off, redissolved in the solvent and reprecipitated several times in order to be certain that the red color is due to the fat alone and not to any of the insoluble stain which may have been accidentally caught in the suspension. This method, although a cumbersome one, has been found most excellent for determining the staining power of various fats and lipoids. The same method may be used when alcohol is employed as a solvent.

In his original communication Klotz expresses a preference for Sudan III, for while he found that Scharlach R stained more intensely, staining with Sudan III was more satisfactory in that the precipitation of crystals from the saturated solution was more easily obviated. In the method which I adopted this difficulty was not encountered. Scharlach R and Sudan III were used interchangeably. No differences were noticeable in fats and lipoids stained with either Scharlach R or Sudan III except differences which would distinguish the original alcohol solutions. Scharlach R stains fats more intensely, the red being much deeper and more pronounced. Fats stained with Sudan III assume more of a deep orange-red color. The staining of fats by these dyes is dependent on the fact that these dyes are more soluble in the fats than in the solution in which they are originally dissolved.

FATTY ACIDS.

Pure oleic, palmitic and stearic acids were employed in the following experiments:

Oleic Acid.—To a definite quantity of oleic acid a small amount of Sudan III in substance was added; so-

lution occurred immediately and the liquid oleic acid was colored a deep red. This color was much more intense than that of the saturated 70 per cent. alcoholic solution. When the oleic acid was filtered into distilled water it separated as deep red opaque globules. Repeated solution and reprecipitation failed to cause the acid to lose its deep red color.

Palmitic Acid.—The acid was dissolved in absolute alcohol and Sudan III added. The solution was now filtered into distilled water. The acid thrown out of solution was not nearly so deeply tinted as the oleic acid and appeared light red in color. If the fluid on which this reddish fatty acid material was floating is heated to about the boiling point the fatty acid melts into globules which take on an intensely red color exactly similar to that obtained when the stain was dissolved in the oleic acid. On cooling the acid separated out as firm red globules. This material collected and redissolved in absolute alcohol was filtered again into distilled water. The fatty acid which now separated out appeared almost white, the slight pinkish color being due to the color of the fluid in which it was suspended. If, however, pure palmitic acid is heated to its melting point and a few particles of the Sudan III are added the acid dissolves the stain and the fluid takes on the deep red color which was noticed in the case of the oleic acid.

Stearic Acid.—The same results were obtained with this acid.

CHOLESTERIN.

This substance obtained in pure form from gallstones was treated by the same method. The first precipitate appeared deep red, but further solution and re-precipitation removed all the color until the final material was almost a pure white. If cholesterol is raised to its melting point and Sudan III in substance is added the fluid cholesterol dissolves the stain and takes on the same deep red color noticed with the oleic acid and fluid palmitic acid. This indicates that only when cholesterol is in a fluid state will it take the stain.

NEUTRAL FATS.

Neutral fats were obtained from both mutton and beef suet. Treated in a manner similar to the other fats, the neutral fluid or semi-fluid fats retained their deep red color after repeated precipitations.

LECITHINS.

Lecithins were prepared from the yolks of eggs and from brains dehydrated with dried calcium sulphate. A quantity of egg lecithin dissolved in a chloroform solution of Sudan III was added to water. The deeply red-colored chloroform fell to the bottom of the vessel. On removal of the chloroform by heat over a water bath, the stain came out of solution and suspended itself in the fluid; the lecithin, on the other hand, assumed a swollen pasty condition like a stiff emulsion which retained a distinct salmon-yellow color. This gelatinous material, which could be lifted out of the solution on a stirring rod, was transferred to fresh amount of distilled water, in which it then dissolved, coloring the aqueous fluid a light yellow pink.⁴ This indicates that lecithin in solution has the power to stain feebly.

In a second experiment with lecithin a quantity of a chloroform solution obtained from dehydrated brain was added to Sudan III solution. The filtered solution was

allowed to evaporate spontaneously and the residue examined under the microscope. Everywhere orange-red globules (lecithin) were interspersed among a yellowish granular material. Lecithin stained with Sudan III, after impregnation in gelatin, also took a salmon-red color. It is obvious, therefore, that lecithin in substance, like the neutral fats, will take and retain the stain.

From these observations it may be concluded that the staining of solutions of the soluble soaps by Sudan III is due to their hydrolysis into fatty acids in which bodies Sudan III and Scharlach R are soluble and which are, therefore, colored by them.

After repeated solution and re-precipitation oleic acid and the neutral fluid fats stained by Sudan III retain their original deep red color. Palmitic and stearic acids and cholesterol, on the other hand, become decolorized by this process and, therefore, can not be stained by these bodies. Lecithin gives a salmon-red color when stained with Sudan III. This is interesting, because it places lecithin, so far as staining reaction is concerned, between the fluid fats and the soaps and solid fats.

According to Michealis,⁵ the staining of fats by Sudan III is purely a physical and not a chemical process, but he admits that the physical properties of a body depend on its chemical character. He considered the union between Sudan III and oleic acid a physical one depending on the property of the unsaturated fatty compound in becoming oxidized. While this may be an explanation for the retention of the stain by the oleic acid, neutral fats and perhaps lecithins containing oleic acid radicals and the non-retention by the palmitic and stearic acids, the fact that other crystalline lipoids like cholesterol act similarly to the crystalline fatty acids suggest that the question as to the staining of the substance depends on its physical condition entirely.

This is particularly emphasized in the experiments with palmitic and stearic acid and cholesterol, where it was shown that when these substances were transformed by heat to their fluid condition they dissolved the stain immediately. On return to their solid state, however, they refused to retain the dye. Again those fatty compounds which at room temperature are fluids or semi-fluids are those which hold the stain even after repeated solution and reprecipitation.

This explains the cause of the discrepancies in the statements of various authors⁶ as to the staining of fatty acids with Sudan III or Scharlach R.

It is believed that the results obtained warrant the statement that only those compounds of a fatty nature take these stains when they possess the physical characteristics of a fluid or semi-fluid at room temperature; other substances which allow of liquefaction by increase of temperature also react to the dye when in their fluid state. Such substances are butyric acid, oleic acid, triolein, tributyrin and lecithin. Palmitic and stearic acids and their neutral glycerids and cholesterol come into this group when the temperature is above their melting point.

The results show that neither calcium nor sodium soaps, palmitic nor stearic acids will at room temper-

5. Ueber Fett Farbstoffe, *Archiv. f. Path. Anat. und Physiol.*, 1901, cxliv, 263.

6. Daddi (L.): Nouvelle méthode pour colorer la graisse dans les tissus, *Arch. Ital. de Biol.*, 1896, xxvi, 142; Rieder (H.): Ueber die Verwendbarkeit des Farbstoffes Sudan III in der klinischen Mikroskopie, *Deutsch. Arch. f. klin. Med.*, 1897, lxx, 444; Handwerck (C.): Beiträge zur Kenntniss vom Verhalten der Fettkörper zu Osmiumsäure und zu Sudan, *Zeitschr. d. Wissensch. Mikroskopie*, 1898, xv, 177.

4. Koch: *Zeit. f. physiol., Chem.*, 1903, xxxvii, 181.

ature take the stain with Sudan III or Scharlach R, hence the material staining by these substances which Klotz described as occurring at certain stages in calcareous degeneration can not be any of these compounds. In all probability it is a mixture of oleic acid, triolein and lecithin. This agrees with the findings which I have reported as a result of the chemical analysis of aortas in various stages of calcification.

GENERAL CONCLUSIONS.

1. The so-called proteid-soap compound of Klotz is not a true chemical combination, but a mixture made up mainly of fatty acids with small amounts of calcium soaps and globulin.

2. Sudan III and Scharlach R stain substances of fatty nature only when they are in fluid or semi-fluid. As oleic and butyric acid, triolein, tributyrin and lecithin are in this state at room temperature, they form a group to which must be limited the application of micro-chemical methods.

THE METHOD OF DETERMINING THE TOTAL AMOUNT OF THE FECAL BACTERIA BY WEIGHT AND ITS CLINICAL SIGNIFICANCE.*

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PHILADELPHIA.

The most accurate method of estimating the total amount of bacteria in the feces is that devised by Strasburger,¹ which determines the actual dried weight of all the bacteria whether living or dead.

Attempts to form an idea of the proportion of bacteria in the feces by plating a given quantity and then counting the colonies is inaccurate for several reasons—principally because a large proportion of the intestinal flora die in their passage through the colon and only a relatively small number of micro-organisms can be cultivated from the stool. Moreover, the different portions of a stool are far from homogeneous, and what is true of one portion will not be true of another.

It must be remembered that results obtained by Strasburger's method are entirely quantitative. No distinction is made between the putrefactive and fermentative bacteria, but the whole bulk of the intestinal flora, living and dead, is measured. Which form of bacteria predominates must be ascertained by other means, chiefly by cultivation, although Ehrlich's aldehyd reaction and the reaction of the feces to litmus may be of some assistance.

Through the use of Strasburger's method various facts have been determined concerning the intestinal bacteria, of which many are new and some are at variance with the received ideas of the physiology of the intestines.

DIET AS A FACTOR.

The diet is probably the most important factor in determining the amount of bacterial growth in the intestines. Foods that are easily digested and well absorbed comparatively early in their passage through the gastro-

intestinal tract leave little or no residue on which the bacteria can live—consequently under such a diet the bacterial count will be low under almost any circumstance. When the diet contains foods which are hard to digest and in which the residue is large the reverse is the case. Consequently the sort of diet must always be taken into consideration in interpreting the amount of fecal bacteria. This is easily seen in Table 1, consisting entirely of normal cases. The following is a summary:

Full home diet gave 18.1 to 20.5 per cent. bacteria.
Full hospital diet gave 18.2 to 21.1 per cent. bacteria.
Semi-liquid hospital diet gave 15.6 per cent. bacteria.
Milk diet gave 13.2 per cent. bacteria.

The great influence that diet has on the intestinal bacteria suggests that the best remedy for excessive bacterial action in the bowel is modification of the diet.

TABLE 1.—NORMAL CASES.

Sex and Age.	Diet.	Stool.	Per Cent. Bacteria	Grams per Diet.	Length of Observation.
1. Male; 38 yrs.	Full home diet	Formed; alkaline; brown.	20.5	7.4	6 consecutive days.
2. Same case...	Same.....	Same.....	18.1	7.4	4 consecutive days.
3. Male; 30 yrs.	Full hospital diet.	Formed; alkaline.	19.1	2.2	2 consecutive days.
4. Same case...	Milk diet; 2 qts. daily.	Formed; alkaline; brown.	13.2	2.2	2 consecutive days.
5. Female; 45 yrs.	Semi-liquid hospital diet.	Formed; alkaline.	15.6	2.2	3 consecutive days.
6. Male; 30 yrs.	Full hospital diet.	Formed; alkaline.	21.1	4.9	Same.
7. Female; 40 yrs.	Full hospital diet.	Formed.....	18.2	2.7	Same.

NATIONALITY AS A FACTOR.

The percentage of bacteria in the feces of American subjects taken from hospital patients and normal persons of the more prosperous class—professional men and private patients—is lower than that given for Germans by Strasburger, who says that he finds that a quarter to a third of the dried weight of the stool is bacteria. This difference in our results is probably to be explained by the fact that the diet is not the same in the two countries. Further investigations in America may show that classes of society who employ coarse foods may show a higher percentage of bacteria. In any event, the proportion of bacteria in the feces is probably higher than has been usually supposed, and this must be considered in estimating the total nitrogen of the stool. The presence of bacteria would furnish an amount of nitrogen that would lead to very confusing results in metabolic work unless the amount of bacteria present was determined and allowances made for this source of error.

BACTERIAL INCREASE.

The bacteria of the feces are *increased*:

1. In conditions in which there is excessive bacterial growth in the stomach, such as achylia with diarrhea; one such case showed 38 per cent. The great influence of a modification of diet in restricting bacterial growth under these circumstances is shown by a case of carcinoma of the pylorus with partial obstruction in a woman of 60 years. There was a great amount of putrefactive and fermentative change in the stomach, with considerable retention but not complete obstruction. Notwithstanding the number of micro-organisms that must have escaped into the bowel through the pylorus, the bacteria in the stool were but 3 per cent., showing that not enough food material came through the pylorus to per-

* Read in the Section on Pathology and Physiology of the American Medical Association, at the Fifty-eighth Annual Session, held at Atlantic City, June, 1907.

* From the Clinical Laboratory of the Presbyterian Hospital in Philadelphia.

1. Strasburger: Ztschr. f. klin. Med., 1902, xlv, 5 and 6; 1903, xlviii, 5 and 6.