REMARKS
ON
A NEW TEST FOR ALBUMIN AND OTHER PROTEIDS.

By JOHN A. MACWILLIAM, M.D.,
Professor of the Institutes of Medicine in the University of Aberdeen.

SALICYL-SULPHONIC acid[1] is a white crystalline substance, readily soluble in water and alcohol. On slow evaporation it crystallises from aqueous solutions in the form of long needles. It gives a deep red colour on boiling with Millon's reagent. As regards its constitution and formation, I quote the following (translated) from Beilstein's[2] *Handbuch d. org. Chemie* (2nd edition, vol. ii, p. 969)—Salicyl-sulphonic acid $C_6H_3(OH)(SO_3H)COOH$.

Formation.—By the action of sulphuric anhydride on salicylic acid,[3] or by heating salicylic acid with concentrated sulphuric acid.[4]

Salicyl-sulphonic acid is a remarkably powerful precipitant of proteid matter. It serves as an exceedingly delicate and precise test for the detection of proteids in solution. It acts upon all the classes of proteid bodies—(1) Native albumins (egg-albumin and serum-albumin); (2) derived albumins (acid-albumin and alkali-albumin); (3) globulins (for example, serum-globulin and myosin, also vegetable globulins); (4) fibrin (whether held in solution by dilute alkalies or by neutral salts); (5) proteoses (albumoses, etc.); (6) peptones.

In the case of all these substances salicyl-sulphonic acid at once forms a dense bulky white precipitate. When the proteid present is a native albumin, a derived albumin, a globulin, or fibrin, the precipitate is not redissolved on boiling; on the other hand, it becomes markedly flocculent. But when the proteid present in the solution tested with salicyl-sulphonic acid is an albumose or a peptone, the precipitate redissolves on heating, to reappear on the cooling of the fluid. Hence it is easy by the application of heat to distinguish between a precipitate due to the presence of albumoses and peptones and one formed by the other proteids. The proteid thrown down by salicyl-sulphonic acid is not at the same time coagulated, as is the case with the precipitate caused by nitric acid, etc. This is shown by the fact that the precipitate is readily soluble in a dilute alkali, provided a sufficiency of the alkaline solution be added. Coagulated proteid is, as is well known, insoluble in such a solution. On the other hand, the precipitate formed by salicyl-sulphonic acid is not soluble in weak acids, nor, indeed, in strong acids, unless a large amount of the strong acid (such as nitric) is added.

When fluids containing proteids (for example, blood serum, solutions of egg-albumin, solutions of albumoses and peptones) are freed from their proteid constituents by treatment with a large excess of absolute alcohol (after acidulation, when necessary) and filtration, they entirely fail to give the characteristic precipitate on the addition of salicyl-sulphonic acid. On the other hand, when the precipitate thrown down by the alcohol, after being well washed with alcohol, is redissolved in water and tested, it readily gives the usual proteid reaction with salicyl-sulphonic acid, a white precipitate being at once formed.

In the case of fluids containing proteids other than peptones (for example, blood serum) the proteid constituents can be completely removed by saturation with ammonium sulphate or sodio-magnesic sulphate, which precipitates the proteids. Here, again, the results are of the same character as those described above. The proteid-free filtrate gives no trace of the characteristic precipitate with salicyl-sulphonic acid. On the other hand, the proteid precipitate, when redissolved by the addition of water, gives a very evident and striking reaction on the addition of the reagent.

Such experiments I have performed with various fluids containing proteids—solutions of the proteids of white of egg (albumin and globulin), blood serum, albuminous urine, 10 per cent. sodic chloride extract of flour (containing vegetable globulin, etc.), saline solutions of fibrin, solutions of Witte's peptone (containing albumoses and peptones), and solutions of albumin which have been subjected to the process of peptic digestion (containing syntonin, albumoses, and peptone). The results have been uniformly similar in all cases. Fluids containing proteids among other constituents give a marked reaction with salicyl-sulphonic acid. The removal of the proteid is invariably accompanied by a complete absence of this reaction, the remaining constituents of the respective fluids being entirely unable—in the absence of proteids—to give the characteristic precipitate when tested with salicyl-sulphonic acid.

ON THE DELICACY OF THE SALICYLIC-SULPHONIC ACID TEST AS A MEANS OF RECOGNISING THE PRESENCE OF MINUTE AMOUNTS OF PROTEIDS IN SOLUTION.

In the case of fluids containing minute amounts of proteid, the reaction which salicyl-sulphonic acid gives is an opalescence or cloudiness of the fluid, instead of the copious white precipitate obtained with such fluids as blood serum, etc.

When employed as a delicate reagent for the detection of traces of proteids it is well to proceed as follows: Take a small quantity of the solution (for example, 20 to 30 minims) preferably in a very narrow test-tube,[5] and add to it a drop or two of the saturated aqueous solution of salicyl-sulphonic acid; shake the tube so as to mix the reagent with the solution, and then hold it towards the light and look at it against a dark background. If necessary use two other tubes as control tubes; one containing some of the solution supposed to contain proteid, the other with water and a drop or two of of salicyl-sulphonic acid.

In the case of extremely weak solutions, where there is only a trace of proteid present the opalescence resulting from the addition of the reagent may not be pronounced until a little time (one minute) has elapsed.

When opalescence is declared the tube is then to be heated to the boiling point, so as to demonstrate whether the opalescence clears up on heating or not.

In the case of very strongly alkaline solutions supposed to contain a trace of proteid, it may be necessary to add more of the reagent than usual, as some of it is used up in neutralising the alkalinity of the fluid. And in case of doubt it is well to test the reaction of the fluid with litmus paper after the salicyl-sulphonic acid has been added, in order to make sure that the alkalinity has been completely overcome and the fluid rendered strongly acid, which it ought to be for the proper action of the proteid test.

It is to be specially noted that the cloudiness or opalescence caused by salicyl-sulphonic acid on shaking it up with a weak proteid solution is uniformly diffused over the fluid; it is entirely distinct from such an appearance as that of a few scattered crystals floating here and there in the fluid and soon subsiding. (Such crystals may appear if the liquid under examination contains a considerable amount of certain salts, for example, ammonium sulphate, etc.)

Extremely weak solutions of proteid (albumin and globulin) were obtained by mixing white of egg with a large amount of water or $\frac{3}{4}$ per cent. salt solution.

The dilution was gradually effected through various stages, and the action of a number of tests was compared at each stage. The results obtained at a few of these stages are stated here.

White of egg diluted 2,000 times:—

Boiling after faint acidulation with acetic acid = no change.

Xantho-proteic test (nitric acid, heat, and ammonia) = slight reaction.

Heller's test (cold nitric acid) = slight reaction.

Mercuro-potassic iodide = haziness of the fluid.

Salicyl-sulphonic acid = marked opalescence.

[1] For the first sample I examined I am indebted to my friend, Dr. McKenzie Davidson. It and the subsequent samples were procured from Messrs. Davidson and Kay, Union Street, Aberdeen.

[2] For this reference I have to thank Professor Japp, F.R.S.

[3] Mendius, *Ann. Chem. Pharm.*, 103, p. 45.

[4] Remsen, *ibid.*, 179, p. 107.

[5] I have frequently used small tubes of three-eighths of an inch diameter, and a length of three inches, and have found them very convenient.

White of egg diluted 8,000 times:
Boiling after acidulation = no reaction.
Xantho-proteic test = no reaction.
Heller's test = no reaction.
Mercuro-potassic iodide = no reaction.
Salicyl-sulphonic acid = distinct opalescence.

White of Egg diluted 12,500 times.—Salicyl-sulphonic acid still gives distinct cloudiness when the test tube is compared in a suitable light with control tubes containing (*a*) some of the dilute solution alone, and (*b*) water or salt solution with salicyl-sulphonic acid. Even with higher degrees of dilution an appreciable effect is obtained. But, without going to the extreme limits of the action of the test, let us take the solution obtained by diluting white of egg 12,500 times. The amount of proteid here must be exceedingly small, even when the dilution is performed with salt solution instead of water in order to keep the globulin of white of egg in solution. Taking the percentage of proteid (albumin and globulin) in white of egg as 12.2, we find that the dilute solution above referred to must have contained less than 1 part of proteid in 100,000 parts of the fluid tested.

A number of experiments were also performed with blood serum, in order to determine the relative delicacy of various tests in regard to the proteids of this fluid (serum-albumin and serum-globulin). The results obtained at a few of the stages of gradual dilution may be stated here. Normal salt solution ($\frac{3}{4}$ per cent.) was employed as the diluting fluid.

Serum diluted 1,000 times:—
Boiling after slight acidulation with acetic acid=no reaction.
Xantho-proteic test=no appreciable result.
Heller's test=no reaction at the time; distinct film five minutes afterwards.
Mercuro-potassic iodide=marked opalescence at once.
Roberts's test (saturated salt solution with hydrochloric acid)=marked opalescence on boiling.
Salicyl-sulphonic acid=marked opalescence at once.

Serum diluted 10,000 times:—
Boiling after being rendered faintly acid with acetic acid= no reaction.
Xantho-proteic test=no reaction.
Heller's test=no reaction at the time, nor five, ten, fifteen, or twenty minutes after.
Mercuro-potassic iodide=no reaction.
Roberts's test=no reaction.
Pietrowski's test (copper sulphate with caustic potash)=no reaction.
Salicyl-sulphonic acid=distinct cloudiness especially marked after about half a minute.

The amount of proteid in such a dilute solution is very small. Taking the total proteids of serum as being about 7.50 per cent.,[6] the amount in the serum diluted 1,000 times must be less than 1 in 13,000; and in the serum diluted 10,000 times, less than 1 in 130,000. Further, it can be shown that with even higher degrees of dilution an appreciable result is obtained when the solution is carefully tested with salicyl-sulphonic acid.

On the Use of Salicyl-Sulphonic Acid as a Test for Albumin and Other Proteids in Urine.

Normal urine gives no precipitate on the addition of salicyl-sulphonic acid.

Urine containing albumin or other proteids gives a white precipitate with this acid; and in the case of the proteids ordinarily present in albuminous urine (albumin and globulin) the precipitate does not clear up on heating; on the other hand it undergoes coagulation, becoming markedly flocculent. With albumoses and peptones, as has been already stated, the result is different; the precipitate which these bodies give clears up on heating, to reappear when the fluid cools. Hence the presence of albumoses or peptones in the urine (the so-called peptonuria) may readily be detected by this reagent; and they may be easily distinguished from the forms of proteid (albumin and globulin) present in ordinary albuminuria.

The fact that albuminous urine readily gives a marked precipitate with salicyl-sulphonic acid does not, of course, prove this reagent to be suitable as a test for the presence of albumin in urine.

There are certain questions to be considered:—

1. Is the precipitate really a proteid one, or is it due to some other substance present in albuminous urine?
2. If the precipitate is a proteid one, is it always obtained when proteid is present in the various abnormal conditions of urine which may have to be examined?
3. Is a similar precipitate given by any other possible non-proteid constituent of urine?
4. How does this test compare as regards delicacy and precision with those ordinarily employed?

I shall deal briefly with these questions *seriatim*:—

1. Is the precipitate really a proteid one, or is it due to some other substance present in albuminous urine?

The fact that salicyl-sulphonic acid gives no precipitate with normal urine tends to show that the ordinary constituents of urine are not the cause of precipitation in albuminous urine.

When albuminous urine is deprived of its proteid constituents, for example, by saturation with ammonium sulphate and filtration, the proteid-free filtrate gives no precipitate whatever when tested with salicyl-sulphonic acid (the filtrate may be freed from excess of ammonium sulphate by dialysis).

On the other hand, the proteid precipitate thrown down by ammonium sulphate when washed with saturated solution of the salt, and redissolved in water readily gives an abundant precipitate when tested with the acid in the usual way; and the presence of proteid is confirmed by other proteid reactions.

Again, the fact that the precipitate thrown down by salicyl-sulphonic acid in albuminous urine is of a proteid nature can be shown in this way: the reagent is added to some of the urine, with the result that a precipitate is at once formed. The urine is then filtered, and the precipitate, after being thoroughly washed with a dilute solution of the acid, is redissolved by the addition of a very dilute alkali (for example, 0.1 per cent. KHO solution). The solution so obtained—the redissolved precipitate—is then examined by various well-known proteid tests, and found to give characteristic results, demonstrating the presence of proteid.

Again, in the case of urine containing albumose, the precipitate caused by salicyl-sulphonic acid may be shown to be a proteid precipitate—precipitated albumose. We can remove the albumose by saturating the urine with ammonium sulphate and then filtering off the precipitate. The filtrate is found to be proteid-free: on the other hand, the precipitate when redissolved in water is found to give marked general proteid reactions, and also those peculiar to albumoses. In all the cases that I have as yet examined, the precipitate in urine (with salicyl-sulphonic acid), which cleared up on heating, to reappear on cooling, proved to be due to the presence of albumose.

Further, in the case of urine containing albumose, if the precipitate caused by salicyl-sulphonic acid be filtered off and redissolved in a dilute alkali, it is found to give the reactions of albumose.

Similar results are obtained with normal urine to which albumose or peptone has been added, with the difference, of course, that the peptone is not removed by saturation with ammonium sulphate, but remains in the filtrate, while the albumose is separated by filtration.

It is clear, from these experiments, that both in the case of urine containing the ordinary heat-coagulable proteids (albumin and globulin), and in the the case of urine containing proteids that are not coagulable by heat (albumoses and peptones), the precipitate thrown down by salicyl-sulphonic acid is of a proteid nature—it is a precipitate of the proteid present in the urine. The results obtained in albuminous urine are in complete agreement with those got with white of egg and blood serum. They make it plain that the proteid is what gives the precipitate, and that the ordinary constituents of urine are entirely unable to give such a result.

2. If the precipitate is a proteid one, is it always obtained when proteid is present in the various abnormal conditions of urine which may have to be examined?

I have examined a large number of samples of urine containing albumin in different conditions, for example, acid

[6] Hammarsten, "Ueber das Paraglobulin," *Pflüger's Archiv*, 1878. Ox serum was used.

and alkaline urine, urine rich in phosphates (deposit), in urates (deposit), mucin (deposit), sugar, bile, etc. In every case I was able without difficulty to recognise the presence of albumin by means of salicyl-sulphonic acid.

The most noteworthy point was that when the urine is very strongly alkaline, as in the case of urine which has undergone an extensive alkaline fermentation, it is necessary to use much more of the reagent proportionately to the amount of the urine tested than in ordinary cases. This is because—as in the case of acidulation by other acids (for example, nitric)—it requires a larger amount to render the urine acid, a portion of the reagent being used up in combining with the alkali present. (This is accompanied by marked effervescence when the urine has undergone ammoniacal decomposition.) And until more acid has been added than is required to combine with the alkali, the albumin is not precipitated. Of course one can economise the reagent in this case by using a smaller amount of urine, so that the relative proportion of acid is greater than the usual. And one can easily make sure that a sufficiency of acid has been used by testing the reaction of the fluid—which now ought to be markedly acid.

It is only, however, when the alkalinity of the urine is very strong that any special precaution is necessary, as with slighter degrees of alkalinity precipitation of the albumin occurs readily on the addition of a drop or two of salicyl-sulphonic acid, as described when dealing with solutions of egg-albumin.

3. Is a similar precipitate given by any other possible non-proteid constituent of urine?

I have examined a large number of varieties of proteid-free urine, and have as yet failed to find any other constituent which gives the characteristic proteid reaction with salicyl-sulphonic acid. The following results may be stated:

Urine which is turbid from precipitation of phosphates clears up on the addition of this reagent, just as with nitric or acetic acids.

Urine containing excess of urates gives no precipitate with salicyl-sulphonic acid.

Proteid-free bilious urine gives no precipitate. Bilious urine, containing albumin as well, gives no precipitate when the albumin has been removed by means of alcohol, etc.

Ox bile was also examined with reference to the possible influence of the reagent on bile salts, etc. The bile was treated with a large excess of absolute alcohol for a number of days, so as to precipitate the bile mucin, and then filtered. The filtrate was evaporated to dryness, and after being washed with alcohol the dry residue was redissolved in water. The solution so obtained was still of a bilious colour; it contained bile salts, as shown by its powerful precipitant action on solutions of acid albumin, etc. With this solution, containing bile salts and pigment, salicyl-sulphonic acid gave no precipitate.

As regards the possible influence of the presence of a large amount of mucin in the urine, I have seen no reason to believe that such would prove a source of error when the test is performed as described in the next section. Moreover, it is probably only in the case of alkaline urine, when there is at the same time a marked irritation of some part of the urinary passages, yielding a greatly increased mucous secretion, that the amount of mucin in the urine can be sufficient to come into question at all. But in such conditions of the urinary tract the detection of a trace of albumin is probably of no significance.

I have examined solutions of mucin in lime water (obtained from the vitreous humour, which is rich in mucin), and have seen very little change on strongly acidifying such a solution with salicyl-sulphonic acid—certainly nothing like the marked effect of acetic acid. Proteid-free urines of persons taking various drugs have not given any precipitate with salicyl-sulphonic acid (the urine was in each case proved to be proteid-free by other tests, sometimes applied after the urine had been concentrated, in order to augment the delicacy of the reactions) after large doses of alcohol, sulphonal, strophanthus, iodide of potassium, morphine, quinine, croton-chloral, after chloroform anæsthesia, etc.

These results are in marked contrast with those obtained with such reagents as a solution of mercuro-potassic iodide (Tanret's reagent); the latter gives copious precipitates in many of those samples of urine.

Solutions of various alkaloids and other substances, many of which give precipitates with some of the known tests for albumin (for example, mercuro-potassic iodide) were examined, and gave no reaction with salicyl-sulphonic acid. Solutions of aconitin, strophanthin, quinine, ergotin, caffein, theine, antipyrin, digitaline, gallic acid, morphine, nicotine, chloral, atropine, strychnine. Balsam of copaiba rubbed up in dilute caustic potash solution becomes markedly turbid on the addition of nitric acid, but shows no change with salicyl-sulphonic acid.

With normal urine, concentrated by evaporation to one-half its original bulk, salicyl-sulphonic acid gave no precipitate or cloudiness. The cold nitric-acid test, on the other hand, showed a considerable amount of precipitation at the junction of the acid and the urine, simulating the presence of albumin.

It need hardly be remarked that the question of the action of salicyl-sulphonic acid towards possible abnormal constituents, medicinal substances, etc., is one that can only be fully answered by extended observation.

4. How does this test compare as regards delicacy and precision with those commonly employed?

Probably the most common test for albumin in urine is the application of heat combined with acidulation. It is pretty generally recognised that the acidulation (when necessary) should be performed before, and not after, the heating; also that acidulation with acetic acid is greatly preferable to acidulation with nitric acid, since, in the latter case, there is a much greater chance of albumin present in the urine escaping detection by being converted into acid albumin, which is not coagulated by heat.

The difficulties connected with the test by acidulation with acetic acid followed by heat, putting aside the chance of a precipitate of mucin being caused by the acetic acid, are (1) that by inaccurate acidulation (insufficient or excessive) albumin may fail to be detected even when present in significant amount; (2) that in favourable circumstances (the most suitable degree of acidulation and rapid heating) this test may be extremely delicate—excessively delicate as many urge—giving a reaction with a proportion of albumin which is clinically insignificant and apt to mislead. In the case of urine which already is somewhat acid in reaction and consequently requires only slight acidulation, there is no doubt that the careful addition of a suitable amount of acetic acid, followed by a rapid heating of the upper stratum of the fluid is a test of very great delicacy.

On the other hand, the cold nitric acid test (Heller's) has often been found to be defective in precision, allowing albumin, even when present in significant amount, to escape notice. It has been felt by many observers that it is desirable to possess a test intermediate in position between the defective delicacy of Heller's test on the one hand and the extreme delicacy (in favourable circumstances) of acetic acid and heat on the other. Such an intermediate test is afforded by salicyl-sulphonic acid when used in the following way. Add a drop or two of the acid to a small amount of urine (for example, 20 or 30 minims) in a small test tube. Shake the tube quickly and examine immediately. The presence at once, or within a very few seconds, of a distinct cloudiness or opalescence in the fluid indicates the presence of an amount of proteid much smaller than is required to give a distinct reaction with Heller's test (allowing one minute for its development), but greater than is necessary to give a reaction by the acetic acid and heat method in favourable circumstances. This, then, is a test intermediate in delicacy between the cold nitric acid test on the one hand and the most favourable action of the acetic acid and heat test on the other. For example, a dilute sample of urine which gave a very slight film with cold nitric acid in course of a minute, gave an immediate opalescence on being shaken up with a drop of salicyl-sulphonic acid, even when diluted (with normal urine) two or two and a half times.

Again, it can be shown that when used in this way the salicyl-sulphonic acid test is markedly less delicate than acetic acid and heat.

A sample of albuminous urine was diluted with normal urine:

One part of albuminous urine in 200 parts: Acidulation with acetic acid and boiling = distinct opalescence of upper (boiled)

stratum of fluid; salicyl-sulphonic acid=no visible change at once or for a number of seconds; in half a minute there is an evident opalescence, and in one minute it is more marked than with acetic acid and heat.

One part albuminous urine in 400 parts: Acidulation with acetic acid and boiling=slight cloudiness; salicyl-sulphonic acid=no immediate effect nor for a number of seconds, in one minute an opalescence is present, more marked than with acetic acid and heat; this opalescence persists on boiling.

Hence it is evident that when *immediate* opalescence is made the test salicyl-sulphonic acid is intermediate between the cold nitric acid and the acetic acid and heat test. On the other hand, when the presence of distinct opalescence after one minute or more is made the determining point the salicyl-sulphonic acid test gives more delicate results than the heat test (after acidulation when necessary) or any other I have tried—picric acid, mercuro-potassic iodide, hydrochloric acid with saturated salt solution, etc.

The main points to be noticed in regard to the salicyl-sulphonic acid test as compared with the acetic acid and heat test, and the cold nitric acid tests, are:

Its use as an agent of a delicacy intermediate between the other two tests; or, if it should be wished, of greater delicacy than either. Its greater simplicity than the acetic acid and heat test; for with the latter, in the case of urines of various grades of alkalinity, care has to be exercised so as to get a correct degree of acidity, in order to obtain certain results; insufficient acidulation being attended with a danger of the albumin present not being coagulated on heating, and excessive acidulation leading to a similar fallacy by the formation of acid-albumin, especially if accompanied by slow heating, which gives more time for the formation of this substance. With salicyl-sulphonic acid there is no danger of adding excess, as, instead of a drop or two, very many drops may be added without harm. Indeed, the addition of an amount equal to the bulk of urine does not redissolve the precipitate formed. All that need be used, however, is an amount sufficient to render the fluid strongly acid—usually a drop or two.

Further, salicyl-sulphonic acid enables one to recognise the presence of albumoses and peptones, which acidulation and heat or the nitric acid test does not.

The drawbacks of the cold nitric acid test, in addition to its insufficient delicacy, are pretty well known—the possible precipitation of acid urates, resinous matters (copaiba, etc.), albumoses, etc. It is true that heat clears up such precipitates, but the application of heat—to the extent necessary to cause a precipitate of albumose to redissolve—is impracticable without involving a mixture of the strong nitric acid and the urine, and consequently a danger of albumin present being redissolved also.

In the case of mercuro-potassic iodide (a very delicate precipitant of albumin) there is the complication of its precipitating many alkaloids, bile-salts, urates in certain circumstances, etc., in addition to albumoses and peptones. And though subsequent heating may clear up the precipitate, it does not differentiate between the various substances. And unless the proportion of the reagent present is a suitable one, the precipitate in some cases fails to clear up, and so simulates albumin. With picric acid, in addition to a much lower degree of delicacy, similar difficulties are present.

To conclude: the method of testing for proteids by salicyl-sulphonic acid may be briefly stated. Take a small amount of urine (for example, 20 minims), preferably in a very small test-tube, and add a drop or two of a *saturated* watery solution[7] of the reagent. If the urine is strongly alkaline, an extra drop or two of the acid should be added, and if no opalescence or precipitate occurs it is well to test the reaction with litmus paper, and make sure that the urine has been rendered strongly acid. On adding the reagent, shake the tube quickly so as to mix its contents. Then examine at once. The occurrence of an opalescence or cloudiness *immediately or within a very few seconds* (for example, 2 to 3 seconds), is a test for proteids intermediate in delicacy between the cold nitric acid test on the one hand, and the acetic acid and heat test (in favourable circumstances) on the other. The development of an opalescence some time after (for example, ½ to 2 minutes), is a more delicate test than even acetic acid and heat, and shows the presence of minute traces of proteid, which are probably insignificant from a clinical point of view as a rule.

Next heat the tube to the boiling point. If the precipitate or opalescence is caused by the ordinary "albumin" (albumin and globulin) commonly present in albuminous urine, it does not disappear on heating, but, on the other hand, becomes markedly flocculent. But if the precipitate or opalescence is due to the presence of albumoses or peptones, it clears up on heating (before the boiling point is reached) and reappears when the tube cools.

[7] It is important to notice that the solution must be a saturated one in order to obtain the best results.

LECTURES
ON THE
PATHOLOGY OF TUBERCULOUS DISEASES OF BONES AND JOINTS.

Delivered at the Royal College of Surgeons of England.

By W. WATSON CHEYNE, M.B., F.R.C.S.,
Hunterian Professor; Surgeon to King's College Hospital and to the Paddington Green Children's Hospital.

Lecture II.

[*Concluded from page 795.*]

Experiments with Tubercle Bacilli on Guinea-Pigs and Goats.—Previous Experimental Work.—Spontaneous Bone and Joint Disease in the Lower Animals.

B. Experiments on Guinea-Pigs.

Two similar experiments were performed on guinea-pigs, in both with positive results, but the processes did not show the same activity as in rabbits, a result like that obtained with tuberculous sputum.

C. Experiments on Goats.

The most important of all the experiments were some which were performed on goats. Müller was the first to employ goats for these investigations. In his experiments he injected the pus from chronic abscesses into the nutrient artery of the tibia, and he selected goats chiefly because the parts are larger. In goats, also, the process appears to remain local for a longer time than in rabbits, and thus the changes in the part can be better studied.

I have performed three sets of experiments on goats. In the first place an emulsion of tubercle bacilli was injected into the nutrient artery of the tibia. Müller had previously performed similar experiments with pus from chronic abscesses, and it seemed to me of importance to repeat them with pure cultivations of tubercle bacilli. The mode of performing the operation, as worked out by Müller, is to expose the upper part of the tibial artery in a goat, just below the origin of the nutrient artery. This is easily done by making an incision on the anterior part of the outer side of the leg, commencing about an inch below the knee-joint. A nerve will be at once seen running downwards superficially to the muscles, and dividing into two branches. Follow the anterior branch and it will lead to an intermuscular space; open up this space, and the vessels will be seen at the bottom of it. Having exposed the artery in this situation it is ligatured and opened above the ligature. The nozzle of the syringe is then introduced into and fastened in the vessel, the nozzle pointing upwards; the material is then injected against the blood stream, and the vessel ligatured as the nozzle is withdrawn. The result of this procedure is that the fluid is forced into the upper part of the tibial artery, and the vessel being blocked below the origin of the nutrient artery it passes along that vessel, and is distributed throughout the bone.

In a second set of experiments the material was injected directly into the joints, and in a third series the injections were made into the substance of the epiphysis. The fluid used was, as before, an emulsion of a pure cultivation of tubercle bacilli.

Experiment XXIII.—The right tibial artery was exposed