this method of arresting the hemorrhage meets with a decided disadvantage in the absence of an adequate resistance to pressure. Notwithstanding the unfavorable mechanical conditions for effective tamponade, this hemostatic agent has given satisfactory results in many cases of traumatic hemorrhage of the spleen, in which it was relied on in arresting the bleeding. It will prove of value if the hemorrhage is venous or parenchymatous, but should never be relied on in bleeding from arteries of any considerable size, that is, in the treatment of arterial hemorrhage. The typical Mikulicz tampon is the one to be employed, as in this case the tampon can be removed with ease, and there is no danger of leaving a part of the tampon in the wound. The tampon also serves the useful purpose of a drain, and should be allowed to remain as long as it is deemed necessary to continue drainage. The size of the tampon can be diminished at any time it is considered safe to do so.

5. *Cauterization.*—The use of the actual cautery in the treatment of traumatic hemorrhage of the spleen has an exceedingly limited sphere of usefulness, if any. It is not reliable, and may inflict serious damage on important adjacent structures. The eschar which it produces interferes seriously with an ideal healing of the visceral wound. For these and other reasons it seems to me that the actual cautery should be resorted to only in exceptional cases as a hemostatic in the treatment of wounds of the spleen.

6. *Marginal Crushing of the Wound and Suturing.*—The most reliable procedure in arresting traumatic hemorrhage of the spleen in all cases in which the wounded organ can be saved is marginal crushing, followed by suturing the crushed margins with catgut. The hemorrhage is arrested the moment the forceps are applied, and the blood vessels in the crushed tissues are speedily obliterated by thrombosis, thus guarding against recurrence of the bleeding, and by uniting the crushed wound margins by suturing with catgut the visceral wound is placed in the most favorable condition for speedy healing and regeneration of the tissues lost by the injury and the operative treatment of the wound.

EXPERIMENTS WITH RENNET AND ANTIRENENE.*

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In 1899 Morgenroth published a paper entitled "The Antibody of Rennet," containing a description of certain work that he had done in support of Ehrlich's chemical theory of the toxin-antitoxin reaction. So far as I know, Morgenroth's work met with universal acceptance without having been subjected to experimental confirmation, nor did I think it worth while to confirm it or make any addition to what Morgenroth had found until the specific precipitates began to attract attention, when their study led me to experiment with antipeptone, antivenene and antirenene.

The antirenene was prepared solely for the purpose of securing the precipitate, but as I found it possessed the power of inhibiting the coagulant action of rennet on milk, I continued to investigate the subject until I found in it a confirmation of what Morgenroth had done, and found myself able to make a few additional contributions to the subject.

Morgenroth's experiments were performed on goats, which were injected with rennet prepared by Witte in Rostock, the activity of this rennet being 1-3,000,000. The sterility of the rennet solution was secured by the addition of iodine (1 c.c. of a 1-10 iodine solution to each 10 c.c. of the rennet solution permitted to stand 1 hour at room temperature), and no abscesses followed its employment. The goat was given increasing doses of the rennet solution administered by subcutaneous injection until 6.5 grams at a dose were administered. After each injection the temperature rose to about 41.4 C., without any disturbance of the general condition or appetite, and without any observable diminution of the body weight. Of the two goats thus treated, one produced a considerable quantity of the antibody, the other attained an extremely small degree of immunity, although both were subjected to exactly the same treatment.

The observation of Schepilewski, that an injection of sterile rennet produced amyloid disease, was not confirmed, as both of the goats remained healthy for more than a year after the close of the experiment.

Morgenroth's studies of the antirenene were made with the blood serum and milk of the goats. The standard rennet solution for testing the antibody consisted of a watery extract of the rennet powder in a 10 per cent. sodium chlorid solution, to which was subsequently added an equal quantity of glycerin and 10 per cent. sodium salt solution. It was found that such a solution contained 2 per cent. of the rennet powder, and could be kept for more than a year and a half unaltered. The presence of the antibody in the serum was tested on cow's milk saturated with chloroform by a long continued shaking in a shaking apparatus and then preserved in the cold.

The method for testing adopted by Morgenroth was as follows: His rennet had the power of coagulating milk in a proportion of 1 part in 3,000,000, but when the milk received an addition of 2 c.c. of the serum of an immunized goat, a proportion of rennet equal to 1-30,000 failed to produce coagulation, though a proportion of 1-25,000 produced a firm clot, so that the serum had the power to neutralize 100 times the coagulating rennet dose, and to thicken the milk 200 times the usual dose was required.

The serum from the other goat that did not attain a high degree of antirenene was so much less active that 2 c.c. inhibited in a proportion of 1-120,000 only. The antibody was extremely unstable, and when preserved with chloroform and kept in an ice chest, its activity disappeared in a few months.

My own experiments were conducted on a different plan and with different animals. Rabbits were chosen for the experiment, and at weekly intervals were given intraperitoneal injections of considerable quantities of the standard rennet solution. The rennet employed is a granular, dry powder of a yellow-cream color, freely soluble in water and physiologic salt solution. My standard solution consisted of a 5 per cent. solution in physiologic salt solution, saturated with chloretone. This preparation was found to be so stable that its strength did not alter on keeping for several months, and it was so active that 1/2,500 of a c.c. produced a firm coagulum in standardized milk in between one and two hours. The strength was, therefore, equivalent to 1-500,000. Each injection of 5 c.c. given to the rabbit contained 0.25 of a gram of the pure rennet.

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The rabbits on which my experiments were made weighed in the neighborhood of 1.5 kilograms each. Morgenroth does not give the weight of his goats, and I am not sufficiently familiar with goats to know exactly what they weigh, but presume an average goat would weigh about 50 kilograms. This being the case, the goats weighed approximately thirty times as much as the rabbits, and basing the calculation on body weight would presumably endure about thirty times as much rennet. My experiments were conducted without any knowledge as to the quantity of rennet administered by Morgenroth to his goats. On subsequently looking it up I found that the largest quantity they received was 6.5 grams. Since the rabbits received 0.25 of a gram at a dose, the goats on the same ratio should have received about 8 grams. This would suggest that the rabbits should have attained a higher degree of immunity and produced a stronger antibody than the goats, supposing their ability to produce the antibody about equal. We find, however, that the rennet with which Morgenroth performed his experiments had a strength of 1-3,000,000, or six times that of my rennet. It is thus evident that in proportion to their weight, my rabbits did not receive nearly as much rennet as Morgenroth's goats, and it is not surprising that I found the anticoagulant powers of their serum considerably less. This is, however, by no means a necessary inference, as Morgenroth showed that of his goats, one produced a serum whose anticoagulant power was 1-25,000, while the other was only 1-120,000, thus confirming what is a familiar observation to every one who has experimented with antibodies, viz., that different animals treated in the same way attain very different degrees of immunity, and very different kinds of antibodies in the blood.

Three rabbits were employed for the experiment. The first died after a single injection. At the necropsy I found an extensive serofibrinous inflammation of the pericardium, pleura and peritoneum, and with more or less pneumonia. The exudates contained several different bacteria. The second animal endured about five injections and then died with identical lesions. In the exudates and blood several different bacteria were found. Both the animals seemed to die early in the night, and when bacterioscopic examination showed that several bacteria were present in the exudates, it was not thought worth while to endeavor to isolate the one responsible for the infection. It is interesting to notice, however, that fibrinous exudates occurred in both these animals.

The third rabbit survived a number of injections of 0.25 of a gram of the rennet and was finally bled to death, the serum being carefully separated for study, and extracts of the different organs of the body made in order to determine whether either rennet or antirenene might be contained in the tissues. For this purpose extracts were made of the medulla oblongata, kidney, liver, pancreas, adrenal, bone marrow, lung, voluntary muscle, stomach, large intestine and small intestine. These extracts were prepared by adding to each fragment of tissue ten times its weight of physiologic salt solution (a gram was considered equivalent to 1 c.c. of salt solution). The organs were finely crushed in a mortar, being reduced to a pulp before the salt solution was added. The mixture was then placed in a test tube and kept in the cold over night. The rabbit, therefore, furnished blood serum, organic extracts and urine for further study.

In order that the investigations might be accurate, it was necessary to standardize the milk, and for this

purpose chloroform and formaldehyd were first tried. The chloroform was found to be less reliable than was hoped, and the formaldehyd was found to diminish the coagulability of the milk. I, therefore, tried chloretone and found that by saturating the milk with this I was perfectly able to protect it against subsequent micro-organismal contaminations, without in any way inhibiting its coagulating power.

As the layer of cream which soon rises and covers the surface of milk kept at the temperature of the body interferes with determining whether the milk has thickened from the presence of the rennet, I found it expedient to use "separator milk" from which the cream was removed. The reaction of the milk affects the action of the rennet; acid milk coagulated much more completely than neutral or alkaline milk. By securing milk fresh from the dairy and immediately saturating it with chloretone, I was able to secure a sufficiently accurate standard, as there was very little variation in the reaction of fresh separator milks, and the saturation with chloretone prevented the development of acidity by bacterial action. The reaction of the milks employed was acid, the acidity varying from 16 to 18 c.c. of normal caustic soda solution being required to neutralize one liter. The difference was so slight that though we tested it carefully it did not in any way seem to modify the action of the rennet, and it is thought not worth while to correct it.

It is well known that the temperature affects the action of rennet, which is much more energetic at high than at low temperatures. Morgenroth preferred to afford his rennet twenty-four hours in which to effect coagulation, keeping the milk in the meantime on ice. Inasmuch as we had eliminated the possibility of any bacterial change in the milk, we were able to keep it at the temperature of incubation, and found that 0.000002 of a gram of rennet added to 10 c.c. of milk invariably produced a firm clot in between one and two hours. We, therefore, adopted the temperature of 37 C. as a standard for the experiments.

In studying the action of the antibody we, therefore, had the following standard conditions:

1. A solution of rennet of which 0.000002 of a gram invariably coagulated 10 c.c. of milk kept at 37 C. in between one and two hours.

2. Milk of a reaction necessary for the appropriate action of the rennet and perfectly preserved from bacterial contamination or alteration either from the production of enzymes or the formation of acids, by saturation with chloretone. (Of this milk, 10 c.c. were used for each test).

The first series was an endeavor to determine whether the rennet injected into the animal was stored up in any of its tissues, and for this purpose 2 c.c. of each of the tissue extracts prepared was added to 10 c.c. of milk and incubated the appropriate length of time. This experiment we made as soon as the extract was prepared.

TABLE 1.—THE FOLLOWING MIXTURES WERE PERMITTED TO STAND FOR 12 HOURS:

10 c.c. Milk + 1 c.c. Extract of	Kidney (freshly prepared)...	=0
" " + " "	Liver	=0
" " + " "	Pancreas	=0
" " + " "	Adrenal	=0
" " + " "	Bone marrow.....	=0
" " + " "	Lung	=0
" " + " "	Voluntary muscle.....	=0
" " + " "	Brain	=0
" " + " "	Blood serum.....	=0
" " + " "	Urine	=0
" " + " "	Large Intestine.....	=0
" " + " "	Small Intestine.....	=+
" " + " "	Stomach	=+

The coagulation produced by the extracts of stomach and small intestine probably depends on

the presence of rennet naturally formed in the stomach. No rennet existed in any of the other extracts, in the blood serum or in the urine. A similar series was made after permitting the sodium chlorid solution to act on the crushed organs for about 20 hours.

TABLE 2.

10 c.c. Milk + 1 c.c. Extract of	Kidney*		=0
" " " " " "	Liver		=0
" " " " " "	Pancreas		=0
" " " " " "	Adrenal		=0
" " " " " "	Bone marrow		=0
" " " " " "	Lung		=0
" " " " " "	Voluntary muscle		=0
" " " " " "	Brain		=0
" " " " " "	Blood serum		=0
" " " " " "	Urine		=0
" " " " " "	Large intestine		=0
" " " " " "	Small intestine		=0
" " " " " "	Stomach		=0

* After 24 hours' maceration.

The next experiments were made for the purpose of determining whether any of the organic extracts contained important quantities of antirenene, and were made with extracts which had been allowed to stand in the cold for about twenty hours.

TABLE 3.—THE FOLLOWING WERE PERMITTED TO STAND FOR 3 HOURS:

10 c.c. Milk + 1 c.c. 20-hour Extract of:		
Kidney + rennet 0.000002 gr.		=+
Liver + rennet 0.000002 gr.		=+
Pancreas + rennet 0.000002 gr.		=+
Adrenal + rennet 0.000002 gr.		=+
Bone marrow + 0.000002 gr.		=+
Lung + rennet 0.000002 gr.		=+
Voluntary muscle + rennet 0.000002 gr.		=+
Brain + rennet 0.000002 gr.		=+
Large intestine + rennet 0.000002 gr.		=+
Small intestine + rennet 0.000002 gr.		=+
Stomach + rennet 0.000002 gr.		=+

Although these experiments were crudely made, they seemed to indicate the absence of important quantities of antirenene in the tissues of the rabbit's body. It might be well to remark that the softest coagulum in the series was that in the tube to which the bone marrow had been added.

The next experiments were made to determine the ability of the blood serum to inhibit the action of the rennet. I find that my method of experimentation differs markedly from that of Morgenroth. Morgenroth used a measured quantity of milk containing an equal proportion of serum and added varying quantities of the rennet. I preferred to experiment by a method more nearly resembling that by which the antitoxic unit is determined in the estimation of the value of antidiphtheritic and antitetanic serums. I, therefore, employed 10 c.c. of milk to which was added exactly the quantity of rennet known to effect its coagulation, and then varying quantities of serum, until I determined the least quantity that would inhibit coagulation.

TABLE 4.—DURATION OF THE EXPERIMENT 3 HOURS. TEMPERATURE 37 C.

10 c.c. Milk + 0.00002 gr. rennet + serum	1 c.c.		=0
" " " " " "	0.7 c.c.		=0
" " " " " "	0.6 c.c.		=0
" " " " " "	0.5 c.c.		=0
" " " " " "	0.4 c.c.		=0
" " " " " "	0.3 c.c.		=0*
" " " " " "	0.2 c.c.		=0†
" " " " " "	0.1 c.c.		=+
" " " " " "	0.09 c.c.		=+
" " " " " "	0.08 c.c.		=+
" " " " " "	0.07 c.c.		=+
" " " " " "	0.06 c.c.		=+
" " " " " "	0.05 c.c.		=+
" " " " " "	0.04 c.c.		=+
" " " " " "	0.03 c.c.		=+
" " " " " "	0.02 c.c.		=+
" " " " " "	0.01 c.c.		=+
" " " " " "	0.001 c.c.		=+
" " " " " "	0.0005 c.c.		=+
Control 10 c.c. milk + 0.00002 gr. rennet + serum			=0

* Soft clot. † Very soft clot.

In this way I showed that the serum effected a distinct inhibition of coagulation, causing the rennet to become inactive in tubes containing 0.5 c.c., or more, of the serum.

The next series of experiments had to do with the specific precipitate, which I expected to find much more delicate, as a means of determining the presence of the antibody rather than the interference with coagulation. Dilutions of the serum and rennet in which the proportions were known, were prepared, placed in small test tubes and kept at the standard temperature. The result was not delicate, as I had anticipated, as is shown by the following table. The quantities of rennet and serum were each diluted with several cubic centimeters of physiologic salt solution, poured together, well shaken, placed in a narrow test tube, and incubated for twenty-four hours:

TABLE 5.

0.00002 gr. rennet + 0.4 c.c. serum		=+
" " " " + 0.3 c.c. serum		=+
" " " " + 0.2 c.c. serum		=+
" " " " + 0.1 c.c. serum		=+ (?)
" " " " + 0.01 c.c. serum		=0
" " " " + 0.005 c.c. serum		=0
" " " " + 0.0033 c.c. serum		=0
" " " " + 0.0025 c.c. serum		=0
" " " " + 0.002 c.c. serum		=0
" " " " + 0.001666 c.c. serum		=0
" " " " + 0.00142 c.c. serum		=0
" " " " + 0.00125 c.c. serum		=0
" " " " + 0.0011 c.c. serum		=0
" " " " + 0.001 c.c. serum		=0

The large quantity of serum required to inhibit the action of the rennet perplexed me, as it seemed not to be in keeping with what was known about other antibodies. It, therefore, occurred to me that the action of the rennet on the milk might take place too rapidly to afford anything but an overwhelming quantity of the serum an opportunity to inhibit it, and that if I would make mixtures of rennet and the antibody in varying proportions and permit them to stand at the temperature adopted for the standard for a number of hours, the rennet might be destroyed by the antibody in very much smaller quantities than was necessary when both were immediately added to the milk. I, therefore, prepared a number of such mixtures and a correspondingly great dilution of rennet, permitted them to stand overnight in the incubator and then added the appropriate quantities to the milk. The result showed that under these conditions the action of the antibody on the rennet was very energetic and entirely confirmed by expectations. Instead of requiring 0.5 c.c. to inhibit coagulation, I now found 0.0005 c.c. to be sufficient, this being the least quantity investigated.

TABLE 6.—TIME, 3 HOURS. TEMPERATURE, 37 C. CONTROL 10 C.C. MILK + (0.00002 GR. RENNET INCUBATED 24 HOURS) =+.

10 c.c. milk + (0.00002 gr. rennet + 0.4 c.c. serum)		=0
" " " " + 0.3 c.c. serum		=0
" " " " + 0.2 c.c. serum		=0
" " " " + 0.1 c.c. serum		=0
" " " " + 0.01 c.c. serum		=0
" " " " + 0.005 c.c. serum		=0
" " " " + 0.0033 c.c. serum		=0
" " " " + 0.0025 c.c. serum		=0
" " " " + 0.002 c.c. serum		=0
" " " " + 0.00175 c.c. serum		=0
" " " " + 0.001425 c.c. serum		=0
" " " " + 0.001225 c.c. serum		=0
" " " " + 0.00111 c.c. serum		=0
" " " " + 0.001 c.c. serum		=0
" " " " + 0.0005 c.c. serum		=0

I was very anxious to determine how much less than this quantity would inhibit the coagulating power of the rennet. But at this point my experiments were interrupted by pressure of other work and the matter was dropped for several weeks. When I was able to return to it again, I found that what Morgenroth had discovered regarding the instability of the antirenene was correct, and that my serum had entirely lost its power, either when added to the milk or when added to the rennet, to interfere with coagulation.

SUMMARY.

The experiments confirm Morgenroth's discovery that the repeated introduction of rennet is followed by the formation of an antibody inhibiting its action. They show that properly prepared mixtures of rennet and antirenene give a specific precipitate, and show, I think for the first time, that the action of the antirenene is infinitely more active when the two are mixed and allowed to stand for some time before being added to the milk than when immediately added to the milk.

This is in thorough keeping with what was observed by Martin and Cherry regarding the interaction of venom and antivenene. I am also able to confirm Morgenroth's observation of the instability of the antibody.

DISCUSSION.

DR. JOSEPH MCFARLAND, Philadelphia—After the use of the chloretone in the milk I found that I was able to keep the milk as long as I liked. Owing to the negligence of the attendant in not cleaning up the bottles on the stand in my laboratory some of them stood for eight weeks without contamination. The milk simply dried up. I have found chloretone one of the best reagents for the preservation of serums, milks, urines and organic extracts from bacterial contamination. Bacteria will not grow in any of these when the chloretone has been added and there is no injurious chemical action.

DR. L. HEKTOEN, Chicago—I would like to inquire whether the rabbit serum has any coagulative power.

DR. J. W. FOSS, Phoenix, Ariz.—Will anything short of a saturated solution of chloretone act as a preservative?

DR. JOSEPH MCFARLAND, Philadelphia—Replying to the first question, normal horse serum contains a body that slightly inhibits the coagulative power of rennin, but rabbit serum does not.

As to the chloretone, I always use the saturated solution because that is the easiest to prepare.

THE PROTOPLASMIC ACTIVITY OF THE RENAL EPITHELIUM,

AS DETERMINED BY THE ELIMINATION OF PIGMENTS INJECTED INTO THE CIRCULATION.*

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INTRODUCTION.

The question of the functional activity of the renal epithelium is ever one of deep interest, both to the physiologist and to the pathologist.

It will be remembered that Bowman's theory concerning the separation of the urine by the kidney was originally founded on anatomic findings entirely and not on experimental evidence. According to this view, the glomeruli separate the watery portion of the urine and the freely diffusible substances, while the specific ingredients of the urine, viz., the urea, uric acid, etc., are secreted by the cells of the uriniferous tubules.

Bowman's theory was replaced for a time by that of Ludwig when the latter investigator and his school showed the close relationship which exists between the circulation of blood in the kidney and the amount of urine separated. This theory held that the separation of urine by the kidney is purely a physical process, the blood-pressure in the glomerular capillaries causing all the ingredients of the urine to filter through into Bowman's capsule in very dilute solution. During the passage of this dilute solution down the uriniferous tubules

the water was supposed to diffuse back from the urine into the lymph, so that the urine becomes more concentrated as it enters the pelvis of the kidney.

Further investigation of the subject soon showed that alterations in the general arterial blood-pressure do not always cause corresponding changes in the amount of urine formed, while the latter may be influenced by conditions which do not affect the former. These facts called the first part of Ludwig's theory seriously into question. The latter part, pertaining to the diffusion of water from the uriniferous tubules into the lymph, was demonstrated to be impossible, as the urine has a greater osmotic pressure than the lymph, and if any diffusion takes place, the water would pass in the opposite direction to that contended by Ludwig.

Heidenhain then put forward a theory which is in part a revival of Bowman's hypothesis in that the specific ingredients of the urine are held to be secreted by the protoplasmic activity of the cells lining the uriniferous tubules. Heidenhain went still further and held that the water and freely diffusible constituents of the urine are secreted by the flat epithelium covering the glomeruli and that these are not merely filtered by a physical process alone, although the influence of changes in the general or local circulation on the flow of urine is fully recognized.

Both of these theories have been modified from time to time, and while there may be minor differences, there are two main views held at the present time as to the functional activity of the kidney:

(1) *The Filtration-Absorption Theory or Modified Ludwig's Theory.*—This holds that all the ingredients of the urine in very dilute solution are filtered through the capillary wall and the epithelium covering the glomerulus, and that the water and chlorids are absorbed by the cells lining the uriniferous tubules, while the urea, uric acid, phosphates, sulphates, etc., pass on to the pelvis of the kidney in more concentrated solution.

According to this view, the epithelium of the uriniferous tubules is not excretive, but is only concerned in absorption. These cells are supposed to have a selective power in absorbing certain ingredients like the chlorids, while other diffusible substances like urea pass on. If this theory be correct, Heidenhain calculated that fully 70 kilos of water must filter through the glomeruli daily.

(2) *The Excretory, Vital, or Bowman-Heidenhain Theory.*—It is contended by those who hold to the other explanation of the function of the kidney that only the water and inorganic salts are separated by the glomeruli. The glomerular epithelium is supposed to be permeable to certain substances and not to others, so that the fluid which collects in the capsule of Bowman contains the inorganic salts in the relative proportion in which they occur in the urine. The cells lining the tubules, and especially those of the contorted tubules, are held to separate the specific ingredients of the urine by an excretory action, i. e., by a protoplasmic activity similar to the secretory activity of other glands.

The main point at issue between these diverse views is whether the renal epithelium is absorptive like that lining the intestine, or excretory in function. If the former is correct, then water and salts are absorbed from the urine into the lymph; if the latter is correct, then the metabolites and possibly some of the salts of the urine are separated by protoplasmic activity from the lymph into the urine as it passes down the tubules.

In trying to determine which of these theories is correct most of the work in recent years has been carried

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