EXPERIMENTAL STUDIES ON INFLAMMATION.

II. Experimental Chemical Inflammation in Vivo.

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In a previous paper the influence of various chemicals upon chemotaxis, *i.e.* migration of cells *in vitro*, was discussed.[1] In the present paper the capacity of these chemicals to produce the other features of inflammation, *i.e.* vascular changes, migration of cells *in vivo*, fluid exudation, and tissue repair, will be considered.

A review of the literature on the subject of experimental inflammation reveals little that throws light on the main problem; namely, what physical, chemical, and physicochemical properties determine that a given substance will or will not produce an inflammatory reaction. No systematic classification has been made of the different substances in regard to their ability to arouse inflammation. Most of the articles have been descriptions of the different cell forms and types of exudates occurring in inflammatory reactions. No definite distinction has usually been made between aseptic and microbic inflammation.

Sollmann[2] alone has attempted to compare chemical structure with a related process, urticaria, but was unable to establish any relation between chemical constitution and urticarigenic action.

Ikeda[3] tested many different substances injected by Cohnheim's method into the frog. He found that normally a certain number of cells migrates into the exposed mesentery from the intact blood vessels within ¾ to 1½ hours, and those substances which decreased the number of cells were therefore considered as inhibiting to inflammation, those increasing the number as arousing inflammation.

[1] Wolf, E. P., *J. Exp. Med.*, 1921, xxxiv, 375.

[2] Sollmann, T., *J. Pharmacol. and Exp. Therap.*, 1916–17, ix, 391.

[3] Ikeda, Y., *J. Pharmacol. and Exp. Therap.*, 1916, viii, 101, 137.

The following substances were found to inhibit the cell migration produced by exposure to air: quinine, atophan, mercury perchloride, arsenic, antipyrine, morphine, chloral hydrate, and calcium chloride. There was a slight inhibition when urethane and ethylhydrocupreine were injected. There was no inhibition produced by cinchonine, scopolamine, atropine, strophanthin, colchicum, sodium salicylate, and magnesium sulfate.

The rest of the abundant literature on experimental inflammation, including the extensive studies of Cohnheim, Marchand, Maximow, and others, reveals nothing of significance in relation to our particular problems.

In the present work experimental inflammation in both frogs and mice has been studied.

Method.

Method Employed with Frogs.—Medium sized winter frogs were used, being kept in a cage so designed that there was a constant stream of fresh cold water through part of the cage, and Cohnheim's method with the frog mesentery was employed. The different substances examined were injected into the dorsal lymph sac before pithing the frog, or were dropped on the exposed mesentery after pithing. Both methods were tried with each substance to serve as a possible check.

As a preliminary test of the behavior of substances introduced into the dorsal lymph sac, colored oils, scarlet R in olive oil and Sudan III, were injected into this cavity. The colored drops could be seen on the exposed mesentery 5 to 10 seconds after injection. Methylene blue solution is visible 3 to 5 seconds after injection. Therefore dorsal lymph sac injection is practically the same as intraperitoneal injection, the fluid passing directly from the sac to the peritoneal cavity by way of lymphatic spaces. In the routine work the first examination was made 30 minutes after injection. If there was evidence of inflammation at this time, examinations were made on other frogs at the end of 20, 10, 5, and even 2 minutes after dorsal injection. If the reaction was very slight or absent at the end of 30 minutes, another frog was examined 1 hour after injection. If there was no evidence of inflammation at this time, the substance injected was arbitrarily classified as one producing no inflammation. Normally from three to five cells per low power field will migrate from the blood vessels into the exposed mesentery in ¾ to 1 hour. In warm weather there may be ten or twelve, and when the surrounding atmosphere is very dry and no attempt is made to moisten the mesentery, this may increase to twenty cells per field. The strength of the solutions varied from 0.005 to 20 per cent molecular concentration of the various substances when the formula was known, otherwise the concentrations were percentage by weight.

Method Employed with Mice.—White mice of medium size, weighing about 25 gm., were injected intraperitoneally under aseptic precautions. Each mouse was kept in a separate cage after injection and the different animals were ex-

amined at the end of 1, 12, 24, and 48 hours respectively, depending upon the reaction to the injected substance. At the end of these periods the animal was anesthetized with ether, and kept under surgical anesthesia throughout the examination. After surgical preparation a midline incision was made into the peritoneal cavity; this was opened carefully to avoid injuring the intestines. The intestines were minutely examined for gross lesions, the mesentery for abrasions, areas of dullness, or changes in color or other abnormalities. The different regions were then examined microscopically in the same way as the frog mesentery. It was impossible to make quantitative determinations of the increase in the number of cells in the mesentery and around the blood vessels because of the numerous cells present normally. Qualitative and comparative differences could be easily determined. During examination the mice could be kept under ether anesthesia for several hours if necessary, and the condition of the heart and the respiration carefully watched. The mesentery was kept moist with sterile physiological salt solution warmed to 37°C., when necessary. In all cases 0.25 cc. of a 1 per cent solution (molecular concentration when the formula was known, otherwise concentration by weight) was injected. When other concentrations were used, they are noted.

EXPERIMENTAL.

Sodium Salts.—The Na ion produced no inflammatory reaction *per se*, being tested as the chloride, tartrate, lactate, phosphate (mono-, di-, and tribasic), citrate, bicarbonate, and sulfate. Sodium cinnamate produced a very slight inflammatory reaction in both frogs and mice, if used in strong (15 per cent molecular) solution. Tribasic sodium phosphate caused some inflammation by virtue of its alkalinity. In frogs, sodium iodide caused a fluid transudate when concentrations over 5 per cent were used, but there were only a few cells outside the blood vessels.

Potassium Salts.—These had little more effect than the corresponding sodium salts, and no inflammatory action can be attributed to the K ion. The iodide caused more hyperemia than the other potassium salts, and in mice a slight leucocytic migration was observed, but not in frogs. The other salts, when concentrated (15 per cent molecular), caused some fluid effusion and a slight hyperemia.

Calcium Salts.—No calcium salt caused a marked inflammatory reaction. In frogs, calcium chloride caused a slightly increased blood flow in 25 minutes in concentrations of 10 to 25 per cent, and calcium citrate produced a slight migration of cells after 20 minutes, if concentrations of 10 per cent or over were used. When a 3 per

cent solution was injected into mice there was marked slowing of the blood stream, and in concentrations of 6 to 20 per cent the mice died of convulsions in 10 to 20 minutes. There were no gross lesions of inflammation at this time, and microscopic examination showed a normal mesentery.

Calcium carbonate, tartrate, and cinnamate caused only foreign body reactions. There was some hyperemia in mice injected with calcium lactate, while citrate caused some slowing of the blood and diapedesis, but no leucocytic migration. Calcium cyanide caused no inflammation when 0.25 cc. of a 0.3 per cent solution was injected; when larger amounts or higher concentrations were injected, the mice died within 10 to 15 minutes with no evidences of inflammation.

Magnesium Salts.—None of the magnesium salts caused any change in the frog mesentery, except the citrate; this caused an active hyperemia. In mice a slight hyperemia was observed with strong solutions.

Strontium Salts.—Strontium chloride caused no inflammation in the frog mesentery, but in mice there were some hyperemia and a slight cellular increase. When equal parts of strontium chloride and magnesium sulfate were used together, there was a slight inflammation as shown by hyperemia and three to seven cells per low power field in $\frac{1}{2}$ hour in frogs. Similar slight effects were seen in mice, exceeding always the effects of either salt alone.

Barium Salts.—Barium sulfate and chloride caused no inflammation. When the solid particles were allowed to remain on the mesentery for several hours there was the typical reaction to a foreign body; *i.e.*, accumulation of cells around the foreign substance.

Mercury Salts.—Mercuric chloride caused no inflammation when a 0.5 or 1 per cent solution was injected into mice. When a 3 per cent solution was used there was a decided shrinking of the cells around the blood vessels and the mesentery was streaked with fibrin, but there was no true inflammation at the end of 24 hours. In higher concentrations the animals died within 24 to 36 hours with no evidences of mesenteric inflammation. Mercurous nitrate caused a very slight inflammatory reaction at the end of 1 hour; after 24 hours the mesentery looked red grossly and on microscopic examination there were many leucocytes, round cells, fibroblasts, and large oval or round cells with many nuclei. Mercuric subsalicylate produced no inflammation.

Free Organic Acids.—Lactic acid, nucleic acid, and uric acid caused no inflammation.

Oleic acid caused a decided dilatation of the intestinal and mesenteric vessels when a 5 per cent solution was injected in mice. The mesentery appeared cloudy, grossly. Microscopically there were fibrinous strands deposited around the blood vessels and an increase in the number of cells in the mesentery.

Amino-Acids.—In mice, glycine produced a slight inflammation at the end of 1 hour. With alanine the rate of flow was decreased but there were a few more cells free in the mesentery than normal. Phenylalanine, glutaminic and aminovalerianic acids, and tryptophane caused a slight increase in the number of polymorphonuclear leucocytes in the mesentery. Histidine caused no inflammation, but there was some diapedesis of erythrocytes. Tyrosine hydrochloride caused a dulling of the mesentery on injection; microscopically there were masses of white blood cells infiltrating fibrinous strands, but there was no increase in the amount of blood present. None of these amino-acids caused any inflammatory reaction in frogs.

Tyramine.—This compound was used in concentration of 1 per cent.[4] It was neutralized with 0.01 N NaOH, with phenolphthalein as an indicator (pH 7.7). In frogs it caused no true inflammation, but the white blood cells were clumped together in all the mesenteric vessels in small groups, at the end of about $\frac{1}{2}$ hour, and there were red blood cells caught with the white blood cells, forming thrombi. The rate of flow of the blood was about normal for 25 minutes after injection, then the rate decreased, and became sluggish, and finally stopped. This clumping of the erythrocytes had previously been observed *in vitro*[1] and at that time no explanation could be given. It seems to affect the blood of different species in the same manner, in the *in vitro* work at least.

In mice, tyramine produced marked changes in the mesentery when 0.5 cc. of a 0.95 per cent solution of the hydrochloride was injected. Grossly the blood vessels appeared dilated at the end of 45 minutes. Microscopically there were groups of red blood cells clumped together within the blood vessels, particularly at the bifurcation of the smaller

[4] The tyramine was made synthetically and was provided by Dr. M. T. Hanke and Dr. K. K. Koessler.

veins, though there were some within the arterioles. In the small capillaries there was no circulation of the blood because of thrombi, although occasionally a thrombus would break loose and some few cells pass through, soon to be stopped by another clump of red blood cells. There were a few more extravascular cells than usual, but only the amount found in slight inflammation. At the end of 24 hours the capillaries showed only masses of clotted erythrocytes and there was no circulation in these vessels. In the mesentery there were some young connective tissue cells, some fibrin, and a few polymorphonuclear leucocytes. 48 hours after injection the flow of blood was still more sluggish than normal because of clots present at the bifurcation of the smaller vessels and in some of the capillaries. These were no longer the agglutination thrombi seen earlier but true clots with fibrin, red blood cells, and white blood cells. The mesentery was approximately normal, but there were more fibroblasts than normal and some early connective tissue cells. This is similar to the results obtained with frogs, although in these the predominating cell in the clumps of cells were white blood cells. With the mouse the clot was composed almost entirely of erythrocytes, white blood cells and fibrin being present only late, and in small amounts.

Histamine.—In concentrations of 0.1 to 0.25 per cent histamine caused marked inflammation when injected into the dorsal lymph sac of frogs. A set of observations is given.

9.00 a.m. Injection of 1 cc. of 0.25 per cent solution of histamine.

9.02 a.m. Increased rate of flow with an occasional white blood cell outside the contracted blood vessels.

9.05 a.m. Blood vessels are less contracted, from nine to twenty white blood cells outside the blood vessels per low power field.

9.10 a.m. More cells are outside the blood vessels, from twenty to twenty-five per low power field, the blood vessels are distended, and the rate of flow is increased.

9.15 a.m. White blood cells are scattered through the entire mesentery, some erythrocytes can be seen leaving the small capillaries by diapedesis, and the rate of flow is increased.

9.20 a.m. Frog died; heart stopped beating.

When histamine was applied directly to the frog mesentery the results were similar to those obtained on injection. Stained specimens made at different times showed deposition of fibrin strands

throughout the mesentery, and a thick layer of fibrin covered the mesentery at the end of 10 minutes, with many leucocytes and erythrocytes in the meshes. This action was in keeping with the marked positive chemotaxis that histamine showed in all the *in vitro*[1] work, when low concentrations were used.

In mice, histamine produced an inflammatory reaction at the end of 1 hour when 0.25 cc. of a 0.25 per cent solution was injected. It was found at the end of 15 minutes that the blood vessels were smaller than normal, and on microscopic examination the lumen was much reduced in size, much more so than the gross appearance of the vessel suggested. There were no evidences of inflammation at this time. 45 minutes after injection the mesentery appeared red, and the blood vessels were distended. Microscopically there were some few cells free in the mesentery, many of them being erythrocytes. These were most numerous around the smaller blood vessels, and some were in clumps around the capillaries. At the end of 1 hour, there were many more wandering cells free in the mesentery than before, and there was marked diapedesis of red blood cells. At the end of 24 hours, there was no acute inflammation but there were fibroblasts present throughout the mesentery, and a few polymorphonuclear leucocytes and some erythrocytes. At the end of 48 hours, there were still a few groups of polymorphonuclear leucocytes and an increase in the fibroblasts and young connective tissue cells. There were areas where erythrocytes had accumulated outside the blood vessels; the cell outlines were indistinct and broken. This is to be expected from the results obtained in frogs, although with frogs the reaction was more rapid and severe. *In vitro* work with histamine showed that the leucocytes of different animals react differently with histamine even under the same conditions, so that the present results are not surprising.

Since this work was done, the report of Bloom[5] has been published. He concludes that histamine does not produce inflammation when injected in cats, though there is a transudate which is most marked at the end of 1 hour. In mice the maximum action occurred at the end of an hour. When collodion capsules containing histamine, 1:100 in physiological saline, were placed between the internal and external oblique muscles in cats, there was a marked inflammatory

[5] Bloom, W., *Bull. Johns Hopkins Hosp.*, 1922, xxxiii, 185.

reaction, but this was in no way different from that caused by collodion capsules containing normal saline, though very different from that caused by collodion capsules containing lactic acid, which is negatively chemotactic. When using open glass tubes containing histamine, *in vitro* and *in vivo*, Bloom found no attraction of leucocytes by the histamine; *i.e.*, no positive chemotaxis. His findings are contrary to those obtained by us;[1] and Sollmann[2] found histamine to be one of the most active agents (1:500,000) in producing urticaria, which is a process closely related to inflammation.

Alkaloids.—None of the following alkaloids caused inflammation: morphine and morphine sulfate, codeine and codeine hydrochloride, quinine sulfate and bisulfate, strychnine sulfate, brucine sulfate, caffeine sulfate and citrate, heroine and heroine sulfate.

All quinine salts caused a slowing of the blood stream and fewer cells migrated into the mesentery from the blood vessels 1 hour after exposure to the air, but the difference was so slight that it is impossible to say that quinine inhibits inflammation.

Ethyl Alcohol.—No inflammation was caused in mice in concentrations below 10 per cent, but in concentrations of 5 to 25 per cent there was a slight migration of white blood cells. In concentrations of 25 to 30 per cent there were many white blood cells outside the blood vessels, from seven to twenty per low power field. These were smaller than normal, and the entire mesentery appeared dry. When fixed and stained, there were strands of fibrin present and the mesentery appeared dehydrated.

Ethyl Acetate.—This compound acted like ethyl alcohol, the inflammation being negligible. It was used as a solvent for cantharidinum.

Ether.—Ether acted like ethyl alcohol, but a 1 per cent concentration acted like the 10 per cent solution of alcohol. There was marked dehydration of the frog omentum in higher concentrations.

Urotropine.—Urotropine, or hexamethylenamine, caused a mild inflammation in frogs with concentrations of 5 per cent at the end of $\frac{1}{2}$ hour if the media was acid. When the mesentery was neutral or alkaline there was no reaction.

Parazol.—This substance (crude dinitrodichlorobenzene)[6] in concentrations of 1 per cent or less caused a slowing of the blood stream of the frog omentum and then in many instances the heart stopped

[6] Wells, H. G., *J. Indust. Hyg.*, 1920, ii, 247.

beating. There were a few white blood cells outside the blood vessels. Stained specimens showed a marked deposition of fibrin through the mesentery, and the cells were indistinct, with poorly stained nuclei. In mice severe typical inflammation was produced by 0.2 cc. of a 0.3 per cent aqueous or oily solution, accompanied by much necrosis. This is somewhat similar to the action of parazol *in vitro*, in which high concentrations cause a negative reaction (chemotaxis) with a necrotizing action, while low concentrations are positively chemotactic.

Papain.—Papain, or papayotin, acted differently in winter and in summer frogs. In the former there was no inflammation when a 20 per cent solution of papain was injected into the dorsal lymph sac or when it was applied directly to the exposed mesentery. When summer frogs were used or when winter frogs were kept in a warm place for several hours, so that the body temperature was over 85°F., there was a definite inflammation on the injection of a 20 per cent solution of papain. This is similar to the results obtained *in vitro*, when the blood of summer or winter frogs was used, the former being positively chemotactic, the latter negatively chemotactic.

In mice, papain, or papayotin, caused a slight inflammation at the end of an hour. There were many white blood cells throughout the mesentery. At the end of 24 hours, the intestines were red, and the mesenteric vessels were distended. Microscopically there were many more wandering cells around the blood vessels than normal and ameboid movements could be seen. In the mesentery there were groups of cells, largely polymorphonuclear leucocytes, though some fibroblasts were present, and in the meshes of fibrin a few erythrocytes. At the end of 48 hours, there were many connective tissue cells with interfibrillar substance, and a few large cells with faintly staining nuclei, epithelioid cells, around the red blood cells. This was similar to the results obtained with summer frogs and is to be expected from the *in vitro* work with the blood of warm-blooded animals.

Cantharidinum.—Cantharidinum (U. S. P.), obtained from Chinese cantharides, caused marked inflammation in the frog mesentery. This drug was dissolved in 5 to 10 per cent ethyl acetate in water. The following is a typical protocol.

8.00 a.m. Injected 1 cc. of a 2 per cent solution of cantharidin into the dorsal lymph sac.

8.02 a.m. Rapid rate of flow of blood.

8.05 a.m. From two to five cells outside the blood vessels per low power field.

8.10 a.m. Marked distention of blood vessels; from nine to fifteen white blood cells per low power field.

8.12 a.m. Increasing number of white blood cells emerging from blood vessels.

8.20 a.m. Many more cells outside the blood vessels, from 70 to 100 per low power field; diapedesis of erythrocytes.

8.30 a.m. Too many cells outside the blood vessels to count; blood vessels contracting slightly.

8.40 a.m. Heart stopped beating.

All the mesenteries were stained and it was found that at the end of 5 minutes after injection the walls of the blood vessels were thinned and strands of fibrin could be followed along the blood vessels. At the end of 15 minutes, the fibrin was present in sheets and many polymorphonuclear leucocytes, red blood cells, and a few lymphocytes were free in the mesentery.

In mice, cantharidinum produced marked inflammation when 0.25 cc. of a 0.5 per cent solution was injected. The mice were unable to stand. Gross examination revealed a yellow exudate at the end of $\frac{1}{2}$ hour. Microscopically there were many polymorphonuclear leucocytes in the mesentery, and fibrinous strands along the blood vessels with erythrocytes in the meshes. At the end of 1 hour, the entire mesentery was covered with polymorphonuclear leucocytes and erythrocytes, and in some places the fibrin was so thick that the cells were hard to see clearly. At the end of 24 hours, there was no exudate present, but microscopically there were groups of polymorphonuclear leucocytes around clumps of fibrin in certain areas. There were many fibroblasts. At the end of 48 hours, the fibroblasts had increased markedly and the polymorphonuclear leucocytes had decreased, the fibrin was diminished, and there were only a few erythrocytes present. 72 hours after injection, there was little evidence of inflammation; the increase in fibroblasts and immature connective tissue alone showed that there had been an inflammation.

Croton Oil.—This substance caused severe inflammation. Some consider that the active principle of croton oil is an acid,[7] others

[7] Bastedo, W. A., Materia medica; pharmacology; therapeutics; prescription writing, for students and practitioners, Philadelphia and London, 2nd edition, 1918, 136.

that it is a resinous anhydride. When 0.25 cc. of a 1 per cent solution in olive oil was injected into mice there was no inflammation at the end of 1 hour, but within 6 hours there were many white and red blood cells in the mesentery and a marked increase in the rate of flow of the blood. At the end of 12 hours, the entire mesentery and intestines were red, and in places the peritoneum was no longer smooth and shining, though there were no definite areas of exudation. There were many cells in the mesentery. At the end of 24 hours, the individual oil drops were surrounded by numerous cells and in many instances by fibrinous strands. There were a few fibroblasts at the outer edges of these clumps of cells. At the end of 48 hours, there were many fibroblasts, many of the polymorphonuclear leucocytes had disappeared, and the oil drops were fewer in number and smaller in size. Young connective tissue cells were present, though not numerous. At the end of 72 hours, there were many fibroblasts and connective tissue cells, but few other evidences of inflammation. These cells were abundant around the traces of oil that were still left. When higher concentrations were used, there was more active, early inflammation, with more polymorphonuclear leucocytes, and many erythrocytes were present in meshes of fibrin.

In frogs similar inflammatory reactions were produced. With solutions over 10 per cent the frogs had convulsions in spite of pithing and at the end of 20 minutes the blood vessels to the mesentery were so torn that it was impossible to examine the tissues under the microscope. Stained specimens at the end of 5 minutes showed large amounts of fibrin in the mesentery and some few polymorphonuclear leucocytes.

Turpentine.—Marked inflammation was induced with concentrations of 0.5 to 5 per cent in olive oil. When higher concentrations were used in frogs the blood vessels ruptured before the animal could be examined. Stained specimens showed strands of fibrin along the blood vessels at the end of 10 minutes, and almost a complete sheet of fibrin over the mesentery at the end of 25 minutes. In mice typical inflammatory reactions were produced.

Jequirity.—A preparation made by grinding the jequirity seeds, adding water, and shaking in a shaking machine for 24 hours caused only a slight inflammatory reaction; indeed there was just about

the number of cells free in the mesentery that is usually found after exposure to air. This was repeated many times with seeds from three different sources, and the same results were obtained, despite the reputation this seed has for producing profound inflammatory changes.

Miscellaneous Substances.—Urethane, urea, creatinine, olive oil, veronal (diethylmalonylurea), yeast vitamine (Harris), olive oil, chloretone, glucose, mercurochrome 220, Witte's peptone, egg albumose, and Armour's peptone caused no inflammation in either frogs or mice. In mice, acetanilide caused a slight inflammation at the end of 8 hours.

The effect of aspirin (acetyl-salicylic acid) was peculiar when the usual amount, *i.e.* 0.25 cc. of a 1 per cent solution, was injected. The animals died within 10 minutes, with no lesions to account for death, and no inflammation was present. When 0.1 cc. was injected, there was no evidence of inflammation at the end of 24 hours.

Scarlet R, purified, caused no inflammation at the end of 1 hour, but 24 hours after injection, there were many erythrocytes and polymorphonuclear leucocytes around the individual oil drops. At the end of 48 hours, there were many fibroblasts, but no true inflammation.

DISCUSSION.

Cohnheim's method for the study of aseptic inflammation gives very satisfactory results for *in vivo* work. Definite quantities can be injected and a microscopic examination made of the mesenteries.

The results may be determined in a definitely quantitative manner by counting the cells migrating out of the blood vessels in a given field, and measuring the increase in size of the blood vessels by the fine screw on the microscope, when frogs are used. With mice, qualitative results only can be obtained; quantitative results are impossible, because of the normal cellularity of the mesentery.

The different types of cells can be detected easily without fixing or staining, but fixing with Zenker's fluid and staining with hematoxylin and eosin make the different cells more distinct; also the changes in the walls of the blood vessels and the amount of fibrin are striking. The migration of the leucocytes through the capillary walls can only be seen distinctly during the life of the animal, before fixation and the subsequent shrinkage.

Dorsal lymph sac injection in frogs is practically the same as intraperitoneal injection; this was clearly seen when colored fluids were injected, as a watery solution of methylene blue, or an oily solution of scarlet R, which make their appearance in the peritoneal cavity almost immediately.

The body temperature of the animal seems to be of importance with certain substances, particularly papain. In the majority of instances, however, it makes no difference. Substances which react differently at different temperatures *in vivo* are found to be those which reacted differently towards the blood of cold- and warm-blooded animals *in vitro*.

CONCLUSIONS.

1. None of the salts tested produce a marked inflammation *in vivo* in concentrations under 10 per cent. Potassium salts and the different citrates produced atypical inflammatory reactions in mice, but not in frogs. There was no true inflammation, however, characterized by blood vessel changes, migration of polymorphonuclear leucocytes and erythrocytes, and fluid exudation.

2. Synergistic action occurs when equal parts of strontium and magnesium salts are employed. There is a change in the appearance of the mesentery without a true inflammation, and this change does not occur with either salt alone.

3. Amino-acids and amines as a class do not produce inflammation, but histamine produces a marked inflammatory reaction in frogs and mice.

4. Tyramine does not cause an inflammatory reaction but has other marked effects; agglutination thrombi occur within the smaller blood vessels, both veins and arteries; in frogs there is a rapid clumping of the white blood cells followed by a true coagulation with strands of fibrin and entanglement of erythrocytes. This is very widespread and often kills the animal within an hour after injection. In mice it is the erythrocytes that clump and coagulation occurs very much later, usually at the end of 24 hours; still later there is complete absorption of the coagulated masses and the mesenteric circulation returns to normal. None of the mice died during the stage of clumping, and the clots never extended up the larger vessels as they did in the frogs.

These effects are similar to the phenomena observed in the *in vitro* work, in which clumping of the cells appeared constantly.

5. Cantharidinum, histamine, and turpentine produced the most rapid and marked inflammation of any substances tried. These substances are all strongly positively chemotactic *in vitro*. The differences occurring when these substances are used in different species is a quantitative rather than a qualitative one, the body temperature being of some importance. Papain acted only in warm-blooded animals; this is consistent with its chemotactic action *in vitro*. The degree of positive chemotaxis varied markedly with the blood employed and in the *in vivo* work the inflammation varied with the species of animal used.

6. Certain substances produced inflammation only some time after injection; this is true of scarlet R and croton oil in weak dilutions. These are not strongly positively chemotactic.

7. Parazol produces an inflammation associated with necrosis of the tissues. This is similar to the results obtained *in vitro*, parazol being positively chemotactic in low concentrations and negative in high concentrations.

8. The exact chemical nature of many of the substances which produce marked inflammation is unknown. This is true of cantharidin, and the active constituents of turpentine and croton oil.

9. All substances which produce marked and rapid inflammation on injection are positively chemotactic, but not all strongly positively chemotactic substances produce inflammation; *i.e.*, calcium compounds, sodium phosphate, etc.

10. Only substances which are positively chemotactic and also soluble in oil seem capable of producing inflammation in animals.