FURTHER STUDIES IN ANAPHYLAXIS.*

IV. *The Localization of Cell and Tissue Anaphylaxis in the Guinea-pig, with Observations on the Cause of Death in Serum Intoxication.*

F. P. GAY AND E. E. SOUTHARD.

(*From the Pathological Laboratories of the Harvard Medical School and the Danvers Insane Hospital.*)

1. The gross lesions of serum intoxication in the guinea-pig. — In 1907 we reported the occurrence of characteristic hemorrhages in guinea-pigs which had received a second properly timed dose of horse serum.[1] We observed such hemorrhages both in those guinea-pigs which died of the intoxication and in those which we killed at various intervals after intoxication. The hemorrhages were not quite constant in occurrence, but were found in one or more organs in thirty-four out of forty-one guinea-pigs autopsied at intervals within the first twenty-four hours after injection. The hemorrhages occurred in a great variety of organs. The most massive hemorrhages, and those most frequently observed, took place in the stomach wall. Thus we found gastric hemorrhages in thirty-two of the forty-one guinea-pigs examined within twenty-four hours of the toxic injection.

Further observations[2] bring our numbers to a total of eighty-six guinea-pigs with hemorrhages in one or more organs, five with doubtful hemorrhages, eleven without hemorrhages in a series of one hundred and two guinea-pigs examined within twenty-four hours of the toxic injection. Fifty-nine of these guinea-pigs (besides three doubtful) showed gastric hemorrhages. We have three new localizations of hemorrhage to report, the brain (three cases), the peritoneum (two cases), the spinal cord (one case).

Hemorrhages were localized in the complete series (1907 and 1908) as follows: Stomach, 59; lungs, 41; heart, 15;

*Received for publication April 15, 1908.

cecum, 9; spleen, 9; adrenal, 8; kidney, 4; muscle, 3; lymph node, 3; brain, 3; diaphragm, 2; liver, 2; pericardium, 2; peritoneum, 2; thyroid, 2; spinal cord, 1.

The occurrence of these gross lesions (in eighty-five per cent of our series of guinea-pigs) escaped the attention of the initial writers on anaphylaxis of this type (Otto in Ehrlich's laboratory, and Rosenau and Anderson in the U.S. Hygienic Laboratory). Otto, in fact, in his first paper[3] specifically denied the occurrence of macroscopic lesions in serum intoxication, and Rosenau and Anderson[4, 5, 6, 7] nowhere state the observation of macroscopic or other lesions in the disease. Lewis,[8] however, working under the direction of Theobald Smith is able to confirm our observations fully and states that Smith, one of the earliest observers of anaphylaxis of this type, had seen such lesions in intoxicated guinea-pigs several years before. Since these publications, there has been neither confirmation nor denial of these findings. Besredka[9] remarks that our structural findings may in the future be of service in clearing up the mechanism of the disease.

As we showed in 1907, these lesions have an important bearing on hypotheses concerning serum intoxication. We showed by an extensive histological study that the gross lesions are only indicators of a manifold series of cell-reactions, involving changes in capillary endothelium, voluntary and cardiac muscle fibers, nerve fibers, and gastric epithelium. As with the gross lesions, the microscopic lesions are distinguished by their focal distribution. This focality extends down to the separate cells, in such wise that single nerve fibers and single muscle fibers are found in a state of extreme or maximal fatty change in the midst of wholly normal fibers.

What is the significance of these lesions? The lesions cannot be regarded as anatomically specific for the serum disease. They belong in the same logical group with the multiple focal hemorrhages of the exanthems, of purpura, and of urticaria. They have much in common with hemorrhages due to local endotheliolysis produced by the

application of such substances as venom. Can the reactions be proved by histological methods to show anything histologically or cytologically specific for the serum disease? This question must be answered in the negative. There is nothing in the lesions of this serum disease which approaches a tubercle or a small-pox vesicle in specificity. The importance of our structural observations does not consist in a demonstration of specific lesions, but in the exact demonstration of one method of producing lesions that are common in various types of disease, particularly in the exanthemata.

2. The cause of death in serum intoxication. — A complete delineation of this kind of pathogenesis would comprise: (1) modifications of tissue during the stage of preparation (anaphylactic phase), (2) the lesions of intoxication (critical toxic phase), and (3) the phenomena of repair and secondary anaphylaxis (reparative and secondary anaphylactic phases). In our former paper we confined ourselves largely to the lesions in the critical phase of intoxication, noting that the primary anaphylactic phase yielded few structural data open to observation. In the present communication we offer a continuation of our structural studies, embracing a more particular account of the cause of death in the critical phase, in the light of which some further observations on the anaphylactic and reparative phases are considered. Nothing is more striking than the sudden death following toxic injection by way of the carotid artery or jugular vein. The hemorrhages in brain, lungs, and heart, produced by such toxic injections, do not admit a purely mechanical explanation, since they are absent in control unused pigs injected with the same amounts of horse serum.

The local fatty changes in the capillary endothelium near the hemorrhagic areas in the heart muscle in these experiments must be closely related to the genesis of the hemorrhages.

But, although striking, these lesions are, in the strict sense, hardly lethal. What is the cause of death in the critical phase?

Hypotheses concerning the cause of death in the critical phase are in the field as follows:

1. *The functional hypothesis.* — Lewis[10] thinks the cause of death in the critical phase depends on "the disordered function of a single organ, or broken coördination between several organs." The hypothesis, as stated, gives no clue to the nature of the functional disorder, or to the organ or organs involved. Lewis leaves in doubt whether the intoxication affects the nervous system or the heart or the lungs, or the heart and lungs through the nervous system. The manifold lesions are admitted, but these lesions are not regarded as pertinent to the occurrence of the functional disorders. It does not appear that this hypothesis is more than a restatement of what occurs in the symptomatic course of the disease, or much more than a blank form for whatever might occur in the process.

2. *The latent brain lesion hypothesis.* — This hypothesis was constructed by Besredka and Steinhardt[11] at the outset of their work. They suppose that the sensitized guinea-pig acquires and retains a latent lesion in its brain. The toxic injection serves to start up (réveiller) the latent brain lesion, bringing about severe disturbances or death. If we substitute for the term lesion (with its structural connotations) the term condition (without such specialized connotations), it is evident that the hypothesis of Besredka and Steinhardt involves scarcely more than the assumption that the symptoms of death in the critical phase are effected through the specially sensitized brain. This hypothesis is, therefore, an advance upon Lewis' functional hypothesis in that Besredka and Steinhardt particularize the supposed source of the disordered functions. Although this hypothesis is not sufficient to explain the lesions of remote organs (such as stomach or cecum since described by us), the hypothesis may contain some truth in so far as it applies to some of the prominent and apparently fatal symptoms. The functional hypothesis of Lewis and the latent brain lesion hypothesis of Besredka have, therefore, much in common; but Besredka indicates

the single organ whose disordered function comes prominently into play.

3. *The hypothesis of specific toxic action upon the respiratory centers.* — There is no doubt that the cause of death is often a respiratory one. From such symptomatic observations, Rosenau and Anderson suggest that we here deal with a poison causing death through the nervous control of the respiration. In order to exclude local effects upon the diaphragm, Rosenau and Anderson[12] showed that the phrenic nerve after death is still excitable, and produces diaphragmatic movements on electrical stimulation after death. Vaughan and Wheeler,[13] dealing with similar considerations in poisoning by white of egg, also maintain that the poisonous substance owes its toxicity to a combination with the cells of the respiratory center. Rosenau and Anderson have since[14] generalized their original suggestion, proposing that "profound chemical changes, perhaps in the central nerve cells, are probably produced by the first injection." In a later paper[15] these writers further maintain that the phenomena of the critical phase are also explained by profound chemical changes, probably in the nervous system. It appears from this work, as well as from our own constant observation (see below) of pulmonary emphysema in those pigs which die rapidly, that the respiratory center, or, at any rate, the nervous respiratory apparatus, is prominently affected by the toxic injection. But, as appears from further considerations, this toxic action upon the respiratory center (or nervous apparatus) cannot be regarded as specific.

Briefly, then, the functional hypothesis of Lewis is scarcely more than a hypothesis in name, since it does no more than restate the problem; the latent brain lesion hypothesis of Besredka lacks a convincing demonstration of either the morphological or the chemical nature of the lesions, either before or after they are "réveillé" by the toxic injection; and the respiratory center hypothesis, as first advanced by Rosenau and Anderson, has considerable evidence in its favor, explains the direct cause of death in most or all

instances, and merely errs in suggesting that the toxic substance is a specific respiratory poison.

Our work to the present time has shown that pulmonary emphysema is a constant feature at autopsy in guinea-pigs dying quickly after the second or toxic injection. We are inclined to regard this emphysema as the effective cause of death in the quickly fatal cases. Of course, the pulmonary hemorrhages and cardiac hemorrhages, which we were able to produce by toxic jugular injections with death in four minutes, are important factors as against possible recovery from emphysema in certain highly intoxicated guinea-pigs.

What is the mechanism of this fatal acute emphysema in the intoxicated guinea-pigs? The emphysema is an expression of death in the inspiratory phase with diaphragmatic spasm. All evidence at hand indicates that the diaphragm is under a constant nervous stimulation of excessive degree during the critical phase following the toxic injection. The probable course of the impulses which produce this hyperstimulation of the diaphragm is by way of the respiratory center in the medulla to the phrenic center in the cervical spinal cord and thence through the phrenic nerve.

Our recent experiments with sensitized guinea-pigs bring out the fact that the more readily the horse serum on the second injection can gain access to the nervous respiratory apparatus, the more certainly will excessive and spasmodic diaphragmatic and general respiratory contractions ensue. The fatalities upon injection of serum into the fluid spaces about the brain (we have preferred the post-orbital route for injection) and into the spaces about the spinal cord (notably in the upper cervical region) are very frequent, and with toxic doses of one cubic centimeter, practically constant.

We shall not here report in detail the results of all our localized inoculations with respect to their differential effects upon the nervous apparatus at large, reserving these findings for special neuropathological consideration. The effects of injections in the vicinity of various parts of the central nervous system are to a great degree differential, and may

prove of some importance in determining special reactions of different regions, especially in the spinal cord. The effect upon the respiratory center is marked and early.

The simplest explanation of this effect is that the toxic factor of the horse serum in these local injections comes directly into contact with the properly sensitized nervous apparatus of respiration, and causes it to work excessively. As is well known, expiration is, under ordinary conditions, a purely mechanical matter; but inspiration is under nervous control. Excessive stimulation of the phrenic center leads to death in the inspiratory phase. Even after opening the thorax and excision of the heart and lungs, intense diaphragmatic contractions may be observed in guinea-pigs dying in the critical phase.

It has so far been impossible for us to determine which portion of the respiratory nervous apparatus is most highly sensitized, or whether any portion is more highly sensitized than another.

It is clear, however, that the hyperstimulation may in some instances be effected over two or three separate conducting elements or neurones. A striking experiment, recently accomplished, consists in the production of respiratory symptoms by touching the vagus nerve (lifted from contact with surrounding structures) of a sensitized pig with a pledget of cotton soaked in horse serum. The same procedure in non-sensitized pigs fails to evoke characteristic diaphragmatic contractions, and salt solution fails to produce the effect in sensitized animals.

This experiment would seem to indicate that the vagus nerve is to some extent sensitized in the general anaphylaxis. We have so far been unable to produce death by this procedure. Fatal issue seems to require immediate stimulation of the medullary or phrenic centers, and, so far, we are unable to report finally which of these centers is the more highly sensitized. Experiments to solve these questions are difficult, because it is desirable to avoid mechanical injury to the centers, but at the same time secure quick absorption of toxic serum by a given center, and distinguish the effects of

the local anaphylaxis from those of anaphylaxis in the rest of the body which the serum speedily invades by blood or lymph.

Our vagus experiments may serve for the present to indicate the line of attack on the problems of local anaphylaxis and to bring out the mechanism of one of the most violent and striking of the symptoms of the serum disease. And our local injections with object of differentially intoxicating different parts of sensitized nervous system (to be reported in detail in a later study) serve simply to bring out the strikingly variable accessibility of the respiratory center, when properly prepared, to a poison injected at different points with relation to the nervous system.

Therefore we desire to substitute, for the theories of the cause of death in the serum disease as so far proposed, and above enumerated, a theory of local anaphylaxis of the respiratory nervous centers. By this we mean a condition induced in the respiratory centers, by means of anaphylactin in the fluid media bathing their cells, such that their elements are excessively stimulated when brought in contact with the toxic or assimilable factor of horse serum. The cause of death is, therefore, an indirect one, being founded on hyperstimulation of the phrenic nerve and cessation of respiration in the inspiratory phase. On this hypothesis the means of avoiding death in the critical phase of intoxication following anaphylaxis would be a therapeutic agent depressing the respiratory centers.

This hypothesis differs essentially from those previously proposed. So far from being due to a disorder of function or incoördination between organs, as proposed by Lewis, the cause of death would seem to be due to an excess of functioning of an apparatus, which is working quite properly in response to the stimulation it receives. The new hypothesis is also more specific than the hypotheses of Lewis and of Besredka. Nor is it necessary to suppose with Besredka a latent brain lesion which is started up once more by the second injection. Rather should we say that a condition had

been induced in these cells, as a result of placing an anaphylactic substance in their surrounding media, such that certain results follow upon injection of a toxic substance. There is an essential difference between the idea of slight irritation followed by severe irritation of qualitatively the same character (as indicated by Besredka's metaphor, "réveiller") and the idea which we support that a substance (anaphylactin) bathing, among other structures, the respiratory nerve cells, has the capacity of altering those cells so that a second substance (normally ineffective) can become effective in stimulating them.

It is obvious that our hypothesis has much in common with any which supposes a specific action upon respiratory centers. And, if Rosenau and Anderson were to ground their hypothesis upon the rendering of these centers specifically accessible to certain toxic substances, we should agree with them. But it seems rather that they suppose that a specific toxine has been produced in the process of anaphylaxis or that a toxine is produced by the union of serum constituents and antibodies,[16] such that the wholly unaltered respiratory center can now be affected by this specific toxine. Whereas Rosenau and Anderson suppose a specific respiratory toxine to be somehow newly produced, we prefer to suppose that the anaphylactin of the first injection has altered certain properties of the cells.

Meantime, the insistence of Besredka upon "latent brain lesions" and of Rosenau and Anderson upon "profound chemical changes perhaps in the central nerve cells" is an indication that these workers fundamentally agree that we must look to the cells for the explanation of much of this problem. It does not seem that Rosenau and Anderson would be forced to hint at profound chemical changes in the cells, if the antibody hypothesis were quite convincing.

A consideration of the cause of death, with cessation of respiration in the inspiratory phase under the influence of respiratory central intoxication, which we can hasten by suitable application of serum near those centers, leads us, therefore, to the conception of a local acquired anaphylaxis or

specific lowered resistance of these centers, under the influence of one substance (anaphylactin) so that they become hypersusceptible to another substance (toxic factor).

An extension of these conceptions to the rest of the body requires separate consideration.

3. Tissue anaphylaxis. — We have so far considered two striking features of the critical phase after the second injection, viz.: The multiple focal hemorrhages frequently found at autopsy within twenty-four hours of the toxic injection and the fatal issue which, when it follows, occurs usually within an hour of the toxic injection. We have not found that either of these features is absolutely constant, although our percentage of hemorrhages (eighty-five per cent) is enough to demonstrate their importance, although the fatal issue seems to vary, roughly at least, with the speed and volume with which the horse serum reaches some portion of the respiratory nervous apparatus. We shall later communicate work on the localization and dosage of respiratory central intoxication.

Is there some broader conception under which these two striking features of the critical phase can be united? Further work upon the histological features of the critical phase confirms the opinion expressed in our paper of 1907 [17] that focal fatty changes are found in very numerous tissues of several different sorts. Some of these changes, especially when several altered cells are in close spatial relation, offer loci of lowered resistance permitting hemorrhages. But it is not at all necessary that the altered cells shall be so grouped as to allow hemorrhages. Take the stomach, for example, in which the most frequent and massive hemorrhages and hemorrhagic ulcerations occur: much more frequent than the hemorrhagic areas in these stomachs are areas in which fatty changes of sharply definite character but without hemorrhages are found.

The focal fatty changes of the critical phase are of more fundamental importance than the hemorrhages, which are

possibly but the mechanical expression of *loci minoris resistentiæ*.

Since our former work, we have studied in particular the tissues of guinea-pigs in various stages of recovery from intoxication and during secondary anaphylaxis, as well as the conditions of second and third intoxications.[18] The result of this work has been to confirm the former findings and to show that the disease is essentially a critical one.

The toxic injection can be said to effect no further disturbance than that of the first hour. Though this could have been surmised from the symptoms, still it might be regarded as likely that some further phenomena would characterize the tissues.

We thought, in particular, that an investigation of the blood picture during the course of the disease might exhibit the tissue reactions more exactly.* This work so far indicates that the same type of reaction — hypoleucocytosis followed by hyperleucocytosis — occurs upon injection of horse serum into normal as into sensitized animals. This fact precludes extraordinary stress being laid at this time upon the leucocyte counts in this disease. The difficulty in drawing blood from guinea-pig ears during the critical phase is an interesting point, which may depend upon contraction of peripheral vessels due to an intoxication of the nervous system.

Although it is true that definite blood alterations do follow the toxic injection, it may be suspected that these alterations have to do merely with the elimination of horse serum and with readjustment of the blood cell supply and that they occur in normal animals in similar fashion after serum injections. A study of the spleens of the guinea-pigs, killed at various intervals of hours and days after the toxic injection, demonstrates that in less than six hours the spleen spaces become filled with polynuclear leucocytes. This tendency is so considerable that the spleens may become visibly swollen. This swelling is only in part due to congestion with

* This was undertaken by Dr. M. M. Canavan of the Danvers Insane Hospital, who proposes to contribute the findings in detail shortly.

blood corpuscles. The intrasplenic leucocytosis is prominent still in guinea-pigs killed at intervals up to twenty-four hours after the toxic injection, but in most cases gradually disappears and gives place to mononuclear phagocytosis during the third day. Some guinea-pigs on the fourth day yield little sign in their spleens of the toxic injection; but in some a moderate leucocyte destruction is somewhat persistent. But although a detailed study is contemplated of the conditions of the peripheral and splenic leucocytosis as well as of marrow conditions, it seems at present, from the orienting examinations already performed, that the relation of these pictures to the process of tissue anaphylaxis is quite obscure and that the body exhibits the same signs of eliminative effort in the blood after the first injection as after the toxic injection. In view of our study of recurrent anaphylaxis [19] in the same animal, these findings become interesting, but it is premature to state that the blood reactions have any relation to the process of anaphylaxis.

These studies may serve to show, therefore, that tissue anaphylaxis is very possibly not at all a function of the blood, detectable by cell changes therein. The reactions, both structural and functional, of the critical phase indicate that very numerous tissues of several orders have been altered in anaphylaxis.

What can be found in these tissues during the stage of anaphylaxis? It will be remembered that the only striking changes which we found in the anaphylactic phase in our former work were certain changes in the peripheral nerves and spinal cord, decidedly less often in nerve tissues above the medulla. These changes consisted in blackenings of scattered myelin sheaths demonstrable in Marchi preparations. The alterations were of two sorts, which we termed respectively linear and nodal changes. The linear blackenings, which exhibit the affected fiber as replaced by a row of black globules and signify a severe injury reparable only by regeneration, were decidedly less frequent than the nodal blackenings. The nodal blackenings, found in the peripheral

nerves characteristically, show a limited osmic acid impregnation confined to the regions of Ranvier's nodes in a given fiber. These nodal changes are regarded as quite consistent with rapid recovery of normal conditions in the affected sheaths. It is possible that conduction is not interrupted by such changes but is altered in ways not now definable.

In addition to these myelin sheaths changes there are other and possibly correlative changes in nerve cells demonstrable by the Nissl method after alcohol fixation. We regarded these as within the limits of technical error, but, so far as reliable, consistent with prodromata toward the well known axonal reaction.

We were not eager to regard these changes as indicative of the latent brain lesions of Besredka or of the profound chemical changes of the nervous system proposed on theoretical grounds by Rosenau and Anderson, and, although we have worked much on this problem since and in particular with the sensitizing euglobulins of Gay and Adler, we are still unwilling to say that our findings represent the essential changes of the anaphylactic phase.

In the first place, we have found similar changes in seemingly normal material, and especially in the diphtheria toxone-paralysis material kindly given to one of us by Theobald Smith. It may be that these changes are simply indices of the extreme lability of nerve tissue, meaning by this lability a capacity for relatively rapid physical alterations of nerve cell and nerve fiber, demonstrable by osmic acid and methylene blue methods.

In the second place, our orienting work with the blood pictures in this disease indicates that to some extent the same species of leucocyte variation is present in the anaphylactic phase as in the toxic phase. So many substances are undoubtedly present in horse serum that the chance of changes effected by other constituents than the sensitizing or intoxicating constituents must be strongly considered. It is possible, therefore, that these anaphylactic nerve cell

and fiber changes are purely incidental to the action of unrecognized constituents of horse serum.

Work is in progress with the relatively pure sensitizing substances (euglobulins of Gay and Adler) to discover what differential effects these substances may bring out.

One further word concerning the relation of the fatty changes and hemorrhages of the toxic phase and possible antecedent changes in the anaphylactic phase. Critics have suggested to us that possibly at some time during the anaphylactic phase fatty changes supervene in various foci in various organs, and that the hemorrhages of the toxic phase are simply mechanical expressions of local acquired weaknesses in the vessels. This hypothesis would suggest that horse serum can in the first instance produce qualitatively the same kind of visible changes which characterize the toxic phase. We have made further examinations of the tissues in large numbers of guinea-pigs. The anaphylactic phase is not characterized by such changes. Using the stomach wall as a histologically pure field for this study, we have yet to find, in any pig killed in the anaphylactic phase, convincing evidence of the presence of intraepithelial fat, vascular fat, or early necroses of epithelium under the influence of anaphylactin.

These statements may be generalized as follows: The essential cytological features of the process of anaphylaxis have yet to be discovered. They do not consist in a minor degree of those changes (focal cytolyses) which characterize the tissues in the toxic phase. The process of anaphylaxis is not a cumulative process, and the toxic phase is not the result of a summation of similar stimuli or effects.

Certain changes, expressive of the extreme lability of nervous structures, have been found in the anaphylactic phase in peripheral nerves and spinal cord. These changes may or may not represent essential features of the process of anaphylaxis. The nervous system is not a clear field, histologically speaking, for the study of minor fatty changes, since these may be the expression of numerous uncontrollable

conditions. So far as the changes discovered have any bearing, the alterations produced by anaphylaxis may be regarded as of a physical nature, permitting speedy rearrangement of various contained substances.

Focal histolyses are not the rule in the toxic phase. Separate cells are more likely to be prepared for intoxication and to succumb thereto (focal cytanaphylaxis followed by focal cytolysis). But the diffuse changes in the stomach wall indicate that focal histanaphylaxis followed by focal histolysis may be the rule under some conditions (effect of gastric juice, etc.). Experience with the critical phase indicates that the cells of certain physiological centers may become highly sensitized (cell group anaphylaxis), and that the functional expression of their intoxication may lead to severe symptoms or fatal results.

4. The reparative phase and recurrent anaphylaxis. — We mentioned in our paper of 1907 that the serum disease might be repeated, and expressed the hope that by suitable repetitions chronic conditions would be produced. This hope has not been realized. The lesions do not necessarily repeat themselves in the site of the original lesions or even in neighboring situations. We have not carried our histological work beyond the reparative stages of tertiary intoxication.

Fresh intoxication in new sites is the rule in secondary or tertiary intoxications. But the repetition is virtually a new instance of the old disease and, certainly in the majority of instances, does not effect a cumulative action upon structures formerly attacked.

We hoped to find in the stomach instances of fresh intoxication superimposed on the remains of old and to discover that recurrent anaphylaxis would emphasize that of the sites formerly sensitized. The stomach seemed to be a likely seat for this cumulative anaphylaxis imagined by us, because of the tendency of the stomach to yield focal histolyses instead of the cytolyses of numerous other organs.

We thought that the new areas of local anaphylaxis might

to some extent coincide with or overlap the old areas of histolysis and lead perhaps to chronic ulceration. But, so far, the conditions have not fulfilled such preconceptions.

This branch of the work, therefore, brings out indirectly the features of the anaphylactin hypothesis. It is the habit of the anaphylactin to sensitize focally certain cells or cell groups. The same anaphylactin in recurrent anaphylaxis is disposed to a similar focal sensitization in which, however, fresh elements are affected.

In those cases of cell group anaphylaxis, after which intoxication expresses itself largely in a functional manner (as in local respiratory center anaphylaxis), a repetition of similar toxic effects can be produced. There was no tendency for repeatedly intoxicated pigs to diminish the severity of their respiratory symptoms. It is possible that, on this line of attack, cumulative effects could be eventually demonstrated in certain centers. It is also possible that separate cells of the respiratory centers are always newly involved in recurrent anaphylaxis and repeated intoxication, and that the toxic effects are not due to activities of the same individual members of the cell group involved. These hypotheses, however, evidently transcend the range of immediate proof.

CONCLUSIONS.

The results of this work are in part confirmatory of our previous results and consist in part of novel data.

Eighty-five per cent of guinea-pigs which, after sensitization with horse serum and intoxication by a second dose of horse serum, die in the critical phase or are killed within twenty-four hours of the second injection, exhibit macroscopic hemorrhages in one or more organs. The stomach leads the other organs in frequency of involvement (fifty-eight per cent); the lungs stand next (forty per cent). Three unusual localizations of hemorrhage, not noted in our previous paper, are brain, spinal cord, peritoneum.

The cause of death, when it occurs, is respiratory. Respiration ceases in the inspiratory phase and shows itself anatomically and histologically as emphysema. Death does not

occur, as a result of this disease, except in a critical phase which occupies at most one hour.

The most striking functional feature of the critical phase, after the second or toxic injection of horse serum, is severe diaphragmatic spasm. The spasms are often accompanied by similar shock-like spasms of the accessory inspiratory muscles and of other trunk and limb muscles.

The most rapid deaths are produced by intracarotid, intrajugular, post-orbital, and paraneuraxial injections. The occurrence and rapidity of death in the critical phase, as well as the severity of respiratory symptoms throughout the toxic phase, appear to vary with the nearness of the toxic injections to the respiratory central apparatus.

A new line of research is opened up by the paraneuraxial injections of horse serum in sensitized guinea-pigs. These seem to prove that differential irritative and paralytic reactions can be secured by small localized injections of horse serum adjacent to various parts of the sensitized central nervous axis.

Severe respiratory symptoms can be produced in sensitized (but not in normal) guinea-pigs by local applications of horse serum (not by salt solution) to the exposed vagus. This is interpreted to signify a conveyance of impulses over at least three neurones to the diaphragm, that is, to the medulla, thence to the phrenic center, and thence to the diaphragm. We have not produced death by these vagal applications of horse serum.

To explain these respiratory symptoms, we offer an hypothesis of local tissue anaphylaxis expressed in a relatively specific sensitization of the respiratory centers. We regard as unfounded those hypotheses which consider the respiratory (and other) centers and tissues as unaltered in the anaphylactic or sensitizing phase, and which allege the manufacture of antibodies in the blood serum which later unite with the second dose of horse serum to form new specific respiratory toxines. We regard this change induced in the respiratory centers as of a physical rather than a chemical nature, so far as this distinction is of importance in this connection.

Neither hemorrhage nor respiratory death is an indispensable feature of this disease. Some guinea-pigs show no hemorrhages. Some show slight symptoms. The hemorrhages do not vary in frequency or extent with the severity of the symptoms in all cases.

But all guinea-pigs so far examined in the toxic phase do show focal fatty changes in many tissues of several genetic types. These changes are, in many regions, of an extremely focal character, involving often a single muscle fiber, nerve-fiber, or other cell, as the case may be. The toxic phase is characterized by focal cytolyses of wide distribution. Except in areas of hemorrhage (where local mechanical destruction complicates findings) and in certain diffuse fatty changes in the gastric epithelium (where the local action of the gastric juice may come in play), groups of contiguous cells are not characteristically affected by fatty change: focal histolysis is not the rule.

And, if focal cytolysis (rather than focal histolysis) is the rule in the toxic phase, then it appears that the work of the anaphylactic phase is to sensitize cells in a variable degree (rather than to sensitize several contiguous or regionary cells in a like degree).

BIBLIOGRAPHY.

1. Gay and Southard. On serum anaphylaxis in the guinea-pig. Journ. Med. Research, xvi, 1907, 143.

2. Gay and Southard. Further studies in anaphylaxis. I. On the mechanism of serum anaphylaxis and intoxication in the guinea-pig. Journ. Med. Research, xviii, 1908, 407.

3. Otto. Das Theobald Smith, sche Phaenomen der Serumüberempfindlichkeit. Leuthold Gedenkschrift, i, Berlin, 1906.

4. Rosenau and Anderson. A study of the cause of death following the injection of horse serum. Bull. No. 29, Hyg. Lab., U.S. Pub. Health and Mar. Hosp. Service, Washington, 1906.

5. Rosenau and Anderson. Studies upon hypersusceptibility and immunity. Bull. No. 36, ibid., 1907.

6. Rosenau and Anderson. The specific nature of anaphylaxis. Journ. Infect. Diseases, iv, 1907, 552.

7. Rosenau and Anderson. A review of anaphylaxis, with especial reference to immunity. Ibid., v, 1908, 85.

8. Lewis. The induced susceptibility of the guinea-pig to the toxic action of the blood serum of the horse. Journ. Exper. Med., x, 1908, 1.

9. Besredka. Review in Bull. de l'Inst. Pasteur, v, 1907, 850–852.

10. Lewis. Loc. cit., *vide* general discussion, ad fin.

11. Besredka et Steinhardt. De l'anaphylaxie et de l'anti-anaphylaxie vis-à-vis du serum de cheval. Ann. de l'Inst. Pasteur, xxi, 1907, v, 119.

12. Rosenau and Anderson. v. Reference 4, 14, 15.

13. Vaughan and Wheeler. The effects of egg-white and its split products on animals. Journ. Infect. Diseases, iv, 1907, 504.

14. Rosenau and Anderson. v. Reference 5. Part xii.

15. Rosenau and Anderson v. Reference 7, 97.

16. Gay and Southard. v. Reference 2.

17. Gay and Southard. v. Reference 1, 161–171.

18. Gay and Southard. Further studies in anaphylaxis. II. On recurrent anaphylaxis and repeated intoxication in guinea-pigs by means of horse serum. Journ. Med. Research, xix, 1908, 1.

19. Gay and Southard. v. Reference 18.