THE HISTOLOGICAL CHANGES PRODUCED BY RICIN AND ABRIN INTOXICATIONS.

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PLATES XI–XIV.

I.

The study of the effects of the living pathogenic organisms upon the animal body has been succeeded by an era in which especial attention has been directed to the influence of their soluble products. The researches of Oertel* upon human diphtheria, of Babes† and of Welch and Flexner‡ upon the experimental form of the disease, the latter including the effects of the soluble products of the growth of the bacillus diphtheriæ, and of Flexner§ upon the pathological changes caused by the soluble products of the streptococcus erysipelatos alone and combined with the diphtheria toxin, as well as studies of the toxic substances contained within the dead bodies of the cholera spirillum, have shown that these toxins may be very potent agents of metamorphosis of cell and tissue, and make it more than probable that in most instances, if not in all, they are the chief instruments of bacterial attack.

Bodies similar to the toxalbumins of bacterial origin have been obtained from the higher plants and even from certain normal animal fluids. Thus from the seeds of the castor plant, Ricinus communis, the jequirity bean, Abrus precatorius, ricin and abrin have been

* Oertel, Die Pathogenese der epidemischen Diphtherie. Leipzig, 1887.

† Babes, Untersuchungen über den Diphtheriebacillus und die experimentelle Diphtherie. Virchow's *Archiv*, cxix, 460, 1890.

‡ Welch and Flexner, The Histological Changes in Experimental Diphtheria. *The Johns Hopkins Hospital Bulletin*, ii, 107, 1891.—The Histological Lesions produced by the Toxalbumin of Diphtheria, iii, 17, 1892.

§ Flexner, The Pathology of Toxalbumin Intoxication. *Johns Hopkins Hospital Reports*, vi, 1896.

respectively isolated by Stillmark,* Sidney Martin,† and Hellin;‡ and from the blood serum of several species of eels the principle, ichthyotoxicum, has been obtained by A. Mosso§ and Springfeld,¶ while Kobert|| has found the toxicity of the bites of the true spiders to be due in part to the toxalbumins introduced into the wounds. The toxalbumins in the bodies of the last-named animals bear no definite relation to their poison glands, for in some species the whole body, including the extremities and eggs, are, according to Kobert, bathed in a fluid containing toxalbuminous substances.

All of the substances now designated " toxalbumins " do not belong to the albumins in the strict sense of the term. Some are globulins, others nucleo-albumins, peptones or albumoses, and still others belong to none of these groups. They are the albumin-digesting, starch-converting, glucoside-splitting enzymes of animal or vegetable origin.** Thus the poisons secreted by the poison glands of various kinds of serpents are now classed with the toxalbumins, although they are far from being simple compounds. Weir Mitchell and Reichert†† believed that they had obtained from rattlesnake venom two principles, a globulin and a peptone; but the latter is not a true peptone as physiological chemists now understand the term; it indeed seems more nearly allied to the albumoses.‡‡ The poison yielded by Abrus is likewise composed, according to Sidney Martin,§§ of a globulin and albumose, of which the former is several times more toxic than the latter.

*Stillmark, Ueber Ricin. *Arbeiten des Pharmacologischen Institutes zu Dorpat*, iii, 1889.

† Sidney Martin, The Toxic Action of the Albumose from the Seeds of Abrus precatorius. *Proc. of the Royal Society*, xlvi, 100, 1889.

‡ Hellin, Der giftige Eiweisskörper Abrin und seine Wirkung auf das Blut. Inaug. Diss. Dorpat, 1891.

§ A. Mosso, Die giftige Wirkung des Serum der Mureniden. *Arch. f. exp. Path. u. Pharmakol.*, xxv, 111, 1889.

¶ Springfeld, Ueber die giftige Wirkung des Blutserums des gemeinen Fluss-Aales (Anguilla vulgaris). Inaug.-Diss., Greifswald, 1889.

|| Kobert, Lehrbuch der Intoxicationen. Stuttgart, 1893, 329.

** Kobert, ibid., 706; Hammersten, Lehrbuch der physiologischen Chemie, 3te Aufl. 37, 1895.

†† Mitchell and Reichert, Researches upon the Venoms of Poisonous Serpents. Smithsonian Contributions, Washington, 1886.

‡‡ Sidney Martin, op. cit., 106.

§§ Ibid.

My previous researches on the histological changes caused by the bacterial toxalbumins led me to extend my studies to include those which are induced by the phytalbumoses, ricin and abrin, and later, the alterations in the tissues of the rabbit brought about by the injection of the blood serum of the dog. The last group of experiments were carried out in conjunction with Dr. Ethel Blackwell, and will be reported at another time.

The samples of ricin and abrin used in these experiments were procured in the open market and came from Merck's laboratory. They fulfilled the usual conditions regarding solubility, coagulating temperature, etc., and were highly toxic. For purposes of my experiments they were dissolved in a 10 per cent solution of sterilized salt solution, and when the total quantity was not used immediately, the residue left for a subsequent experiment was preserved by the addition of 0.5 per cent of carbolic acid. Before injection the solutions were diluted with sterile distilled water, so as to render them of an equal density with physiological salt solution.* The animals which served for this study were rabbits, guinea-pigs and mice. They were chosen with some care, many having been raised in the laboratory, and none were subjected to inoculation which did not at the time appear to be healthy. The material to be injected was introduced subcutaneously, intraperitoneally or intravenously. In mice the first method was employed exclusively; in guinea-pigs, the first or the second; and in rabbits, all three severally. The quantity of poison injected varied within narrow limits, as it was my aim in every instance to introduce into the animals an amount which would always prove a certainly fatal dose.

In giving the results of my studies the individual protocols will not be detailed, and only the extremes of dosage and duration of life need be stated. It does not seem possible to make a distinction in respect to the pathology of the process depending upon amount of toxic agent and duration of life where the latter is short and does not exceed a

* A preliminary communication concerning this study appeared in the *Medical News*, August 4, 1894, under the title, "The Pathologic Changes caused by certain so-called Toxalbumins."

week or ten days. On the other hand the more chronic forms of intoxication are likely to be associated with pathological changes which differ very much in degree, if not in kind, from those of the acute cases.

The autopsies upon the dead animals were performed as soon as possible after death, the attempt having been made so to choose the time of inoculation as to bring about death during the day hours. While in the majority of cases this procedure was successful, in some it failed. It was always my aim to obtain the tissues in the fresh state, as the finer histological methods are much less efficient when applied to tissues in a state of even incipient post-mortem decomposition. The changes in the intestinal mucosa in particular are not to be relied upon unless these conditions are fulfilled. In order to obtain trustworthy results the intestine should be hardened without disturbing its mucous surface and with its contents adhering to it.

As hardening agents, alcohol, graded or 95 per cent from the first, bichloride of mercury, formalin, Flemming's and Hermann's solutions were employed. The sections were made in paraffin or celloidin, more often the latter, and stained with hæmatoxylin and eosin, carmine, magenta, safranin, and, for special purposes, Weigert's fibrin stain. In order to ascertain the presence of fat in situations from which it is normally absent, or of estimating its amount where it is commonly present, fresh frozen sections were employed. These served, at the same time, for the study of other forms of degeneration.

The close relationship existing between the bodies ricin and abrin and certain bacterial toxins has already been alluded to. The studies of Kobert made it appear probable that these bodies, although obtained from two entirely different sources, yet possess identical properties. The experiments of Ehrlich* on the production of immunity in mice and guinea-pigs from these substances proved that, although they possess many features in common, yet they are essentially distinct.

The observations of Kobert and his pupils were limited to the action

* Ehrlich, Experimentelle Untersuchungen über Immunität. I. Ueber Ricin. II. Ueber Abrin. *Deutsche med. Wochensch.*, 1891, 976 and 1218.

of these bodies on the blood, and incidentally on such organs as the gastro-intestinal tract, the lesions of which he attributed to the vascular thromboses believed to exist there. Stillmark failed entirely to discover lesions in the parenchymatous organs. Ehrlich also considers the pathological changes in the viscera (stomach and intestine) to be the results of vascular disturbances.

My experiments have led me to entirely different conclusions regarding the mode of action of these substances and to consider them as acting upon cells and tissues much like the bacterial toxins. Indeed, while this resemblance is more or less striking, the pathogenic behavior of ricin and that of abrin are, under these circumstances, so nearly alike that except for Ehrlich's important observations I should feel inclined with Kobert to regard them as physiologically identical.

The doses employed varied, in the rabbit, from 0.1 to 3 milligrammes per kilo of body weight, and in the guinea-pig from 0.25 to 1 milligramme per kilo of body weight. The duration of life following inoculation was subject to fluctuation. The shortest period observed was in the rabbit (3 milligrammes ricin per kilo) which succumbed as early as in six hours, and the longest in the guinea-pig (1.5 cc. of a 1 to 200,000 solution) which survived about 6 weeks. A few animals after a varying period of illness recovered entirely and were protected from the effects of subsequent inoculations.

The picture presented by animals which have been subjected to inoculation with either substance differs somewhat and depends upon the dose, and still more upon the mode of introduction of the poison. In all cases there is a rise of temperature, averaging about two degrees; diarrhœa is present, the stools being of a bloody character unless the animal succumbs very quickly; the urine is always albuminous and contains tube casts and in rare cases red blood corpuscles; severe convulsions, opisthotonos and great general weakness, preceded by a fall in temperature, are likely to occur before the end.

The gross pathological changes after intravenous inoculation consist, first, of punctiform hæmorrhages, most abundant in the peritoneal cavity, where they are scattered over the omentum and beneath the serous covering of the intestine, mesentery and abdominal parietes.

Similar ecchymoses, smaller in number, are found beneath the serous covering of the solid abdominal organs and of the heart and lungs. The peritoneal cavity contains, in a large majority of instances, a great excess of fluid, which is often opaque and hæmorrhagic. As much as 100 to 150 cc. of such fluid may be present. On the intestine, in which the fluid presents a bloody aspect, the subserous hæmorrhages are very numerous. Several pregnant female animals were used for experimental purposes, and in all free hæmorrhages had taken place into the fœtal membranes. No gross lesions of the blood-vessels were discovered anywhere in the abdomen; the fluid and accompanying blood corpuscles evidently passed out of the injured vessels by transudation and diapedesis. On the other hand no excess of fluid existed in either the pleural cavities or the pericardium.

The lymphatic glands generally showed enlargement, they were softened and congested or hæmorrhagic; the mesenteric glands more especially were swollen. One of the most striking features was the condition of the intestinal mucosa. In the case of very rapid death (6 hours) the intestine was filled with opaque, almost fluid contents much resembling cholera stools. The mucous membrane, as a rule, was pale; Peyer's patches were much swollen, elevated and of a white color. In the other case the appearances are usually somewhat different; while the intestine is always distended, the contents are semi-fluid, tinged with blood, and the general surface of the mucosa is greatly congested and beset with pin-point hæmorrhages. Peyer's patches become very prominent and deeply congested, the intervening mucosa showing less swelling. The whole picture suggests the *surface reticulée* of the typhoid intestine. Actual ulceration, contrary to the views expressed by other writers (Kobert, Ehrlich), was never found. The fact that these observers apparently did not take the precaution of confirming their opinions by microscopical examinations necessarily throws doubt upon the existence of such lesions. The mucous membrane of the stomach was congested in all cases. In a few instances superficial losses of substance in the mucosa were noted. Around these signs of reaction were missed. It should be noted that in one rabbit (No. 8) there existed in the stomach a congested patch the size

of a quarter of a dollar, over which the epithelium was entirely necrotic.

The spleen was uniformly swollen, of dark color and of less firm consistence than normal. The swelling was apparently of the splenic pulp. The liver was always dark in color and hyperæmic. Focal lesions, resembling the areas of necrosis described first by Welch and Flexner* in experimental diphtheria and now known to occur in many kinds of infection as well as in several intoxications, were always present. These were in some cases just beneath the capsule, in others within the deeper substance of the liver. They were most evident to the naked eye when in the former situation and, as often happened, when they were surrounded by a zone of hyperæmia or hæmorrhage. The other organs, save more or less hyperæmia, appeared to the naked eye normal.

Subcutaneous inoculation produced, besides the lesions mentioned, also local effects. In very acute cases the subcutaneous tissues presented an œdematous condition, which was often hæmorrhagic in character. The local lymphatic glands were now much enlarged and reddened. In more protracted cases a subcutaneous node formed, followed by necrosis, and eventually by sloughing of the skin.

II.

Lymphatic Glands.—The histological study may begin with a consideration of changes in the lymphatic glands. The extent of injury to a particular group of glands depends largely upon the mode of access of the poison, for when this is injected directly into the blood all the glands may be presumed to receive an approximately equal share; when, however, the poison is introduced beneath the skin, the nearest glands must receive a much larger quantity. The differences, however, in the extent of the affection of different groups of glands cannot depend upon this factor alone, for even in the instances of vascular distribution, not all the lymphatic glands suffer equally.

The channels of elimination of the poison from the body determine still another avenue of glandular affection in excess of the vascular

* Loc. cit.

form. It has been shown by Stepanoff,* and by Calmette and Delarde,† that ricin and abrin are removed from the body largely through the intestinal tract, and this elimination is accomplished, as I shall show, not without great injury to its constituent histological elements. It would also appear that a second absorption (resorption) from the intestinal tract may take place, in which case the mesenteric glands are more than ordinarily swollen, reddened and softened.

The histological changes in the lymphatic structures are in part productive, but chiefly degenerative in character, and affect the lymphoid cells, the most pronounced feature being death by fragmentation of cytoplasm and karyoplasm. In some lymphatic glands every node is affected, in others the lesions are relatively insignificant. The peri-follicular lymph sinuses and the lymphatic spaces beneath the capsule contain fragmented cells and large phagocytes (macrophages) loaded with metachromatic-staining globular particles. Within these channels, and more frequently in the peripheral parts of the follicles, karyokinetic figures are seen in small numbers. The medullary portion of the gland also suffers in a similar way. The most intense alterations are found in the inguinal and axillary glands in cases of subcutaneous inoculation. In extreme cases nothing remains of the original normal tissue of these glands. The swelling of cells and coincident hæmorrhages obliterate the peri-follicular lymph sinuses; the medulla is permeated with blood; the lymph cells in the follicles are converted into lightly staining nuclear fragments and feebly staining remnants of cell protoplasm (Plate XI, Fig. 1). A fine network of fibrin traverses these areas. The medulla shows no intact cells, but only fragments and altered protoplasm remain. Macrophages are abundant in the follicles and in the similarly affected lymph cords, and appear here, there and everywhere in the medullary part of the gland. Nuclear residues of all forms and sizes and particles of blood pigment are included in these bodies. The blood-vessels are often overcrowded with red corpuscles; coagula of any sort were entirely missed.

* Stepanoff, Études sur la ricine et l'anti-ricine. *Annales de l'Institut Pasteur*, x, 663, 1896.

† Calmette and Delarde, Sur les toxines non microbiennes, etc. *Annales de l'Institut Pasteur*, x, 675, 1896.

Intestine.—Of all the constituents of the intestinal mucosa, the one to feel the most severe effects of the poison is the epithelium. Here again the most marked alteration is the cell disorganization effected through transformation both of nucleus and cell body, although the picture is dominated by the changes in the former. All sorts of intensely staining bizarre forms appear within the protoplasm of the cells, from the minutest particle of chromatin to crescents, imperfect vesicles, agglomerations of particles with rosette and stellate forms and flask-shaped bodies. The protoplasm itself is granular and opaque, partly disintegrated or fused with neighboring cells. Many of the particles of nuclear material, exhibiting the most striking metachromatic coloring by the aniline dyes, have found their way into the lumina of the glands on one side and that of the intestine on the other (Plate XIV, Fig. 10).

The villi in the small intestine also suffer. The cells distributed within their delicate reticulum become increased in number, swollen and finally fragmented. The changes in the vessels themselves, consisting of necrosis of lining cells and circulating leucocytes, form a striking feature. The epithelium covering the villi, but especially that within the crypts, shows mitoses, which, in the latter situation, in cases of less severe injury may be numerous.

Congestion is a marked feature in the gastric and intestinal pathological processes, and hæmorrhage is not uncommon. The blood-vessels in submucosa and mucosa are over-distended, but no relative increase in white corpuscles is discernible. The vessels are not thrombosed; no trace of fibrin can be found in ordinary preparations or such as were stained especially for this material.

Ulceration in the true sense of the term does not exist. On the other hand there has been a free desquamation of necrotic epithelial elements which, together with fragmented cells and what resemble emigrated mononuclear or lymphoid elements, are piled up on the surface of the mucosa, mingling with the other intestinal contents. The whole may form a layer of some thickness, and may resemble superficially an exudate; it shows nothing of the nature of a genuine inflammatory product, and is as devoid of polymorphonuclear cells as of fibrin. It does, however, contain great numbers of bacteria.

The lymphoid tissue of the intestine suffers considerably. The lymphoid cells, which are scattered diffusely, go through the same evolutionary and involutionary changes as those in the collections forming the solitary follicles and Peyer's patches. The patches themselves show an increase in size, due partly to the swelling of the cells, œdema, congestion or hæmorrhage, and partly to the presence of large phagocytic cells and included cell fragments, or sometimes possibly to actual multiplication of the lymphoid elements.

The stomach is affected to a distinctly less degree than the intestine. Congestion is marked; hæmorrhages are relatively infrequent and of small size; necrosis of scattered cells exists to a limited extent; the surface epithelium may desquamate, leaving small foci open to the invasion of various kinds of bacteria already present, which may force their way for a limited distance into the gastric glands.

Spleen.—With some few exceptions it has been found that the bacterial toxalbumins affect the Malpighian bodies far more profoundly than the splenic pulp. It would appear as if in the case of the phytalbumoses the seat of most intense action is reversed. The follicles are, however, not without easily discernible lesions. Necrosis (preceded by swelling) of cells by fragmentation exists, affecting small and large cells, separated by only slightly affected or almost normal lymphoid cells; many of the degenerative products are included by phagocytes (Plate XIV, Fig. 8). These lesions are hardly comparable to those which are met with in the same specimens in the pulp. In these small animals there is every appearance suggestive of the blood within the pulp circulating in free and wide spaces lined with a high endothelium. The walls of the sinuses are very thin and attenuated and contain so few cells that the lesions are found especially in the blood-vessels and only to a slight extent outside of these. The degenerated cells in the splenic pulp are of every kind met with in this situation. As the whole number has received augmentation owing to the disease process, the total injury is much greater than could otherwise be accounted for. Endothelium, leucocytes, mononuclear and polymorphonuclear, red blood corpuscles and lymphoid elements all succumb to the destructive effects of the poison (Plate

XIII, Fig. 7). Globules and fragments of protoplasm and of nuclear substances, and small and large balls of golden yellow pigment, are sometimes found free, but chiefly within cells, many of which exceed in size those observed even in the most pronounced bacterial infections and intoxications. These giant phagocytes—veritable macrophages—are enormously swollen and loaded with an indiscriminate and incongruous mixture of parts of cells, entire altered cells and pigment, to which the metachromatic properties of many of the particles, when stained in safranin or magenta, lend a brilliantly variegated appearance. The ultimate destination of the macrophages is not clear; they are found in various stages of development within the sinuses; soon they occupy the width of a sinus, and in cross sections of apparently sound vessels they are to be found completely filling the lumina (Plate XIV, Fig. 9). The capillaries of the stroma may show only more or less cell degeneration or, what is of more interest, extensive fibrinous thrombi, which in some instances are present. The blood-vessels, except the capillaries, are widely open and contain red corpuscles of normal appearance.

Liver.—The pathological alterations are of two kinds; one only, however, will be considered in some detail. The mere swelling, increased granulation and fatty metamorphosis, which are found in the hepatic cells generally, need not be discussed, as these lesions are found in a great variety of conditions associated with altered nutrition and are well known. One of the common appearances in the liver is brought about by a great dilatation of the intralobular capillaries, which often causes a marked compression of the rows of liver cells. The packing of red corpuscles is very evident, and the individual outlines of these corpuscles may not be distinguishable, thereby suggesting the formation of thrombi of red corpuscles in the sense of Kobert and of Ehrlich. White corpuscles do not appear to be increased in number in the blood in general.

The more specific changes are not distributed equally throughout the entire liver, but are focalized. They affect, also, scattered individual cells and scattered groups of cells. These alterations are attended by necrosis, actual death of the cells, rather than by simple.

nutritive disturbances, as in the diffuse lesions. The initial focal lesion is a swelling of the cells whereby the intervening capillaries may be compressed. Simultaneously the cell protoplasm becomes pale, homogeneous and transparent. The nuclei may still stain, but often only feebly. There is no evidence of pathological fatty metamorphosis at this stage, although the two processes may be associated. Very soon a further transformation of the affected cells occurs, the protoplasm now showing an increase in refraction, thus exhibiting the peculiar appearance to which the name "hyaline" has been applied. An especial characteristic of these altered cells is their capacity to fix acid dyes (such as eosin), which they take up with great avidity and retain with equal persistence. The hyaline change thus described may be limited to a small part of the cell, or it may affect entire cells or groups of cells occupying the whole field of the microscope (Plate XII, Fig. 3).

The behavior of the nuclei varies in different instances, often in the same section of the liver and not infrequently in the same necrotic focus. Perhaps the commonest feature is the loss of staining capacity, such as occurs in karyolysis from various other causes; but nuclear fragmentation or karyorhexis, which is a more striking change, is not wanting. An earlier change than the latter, and probably than either of the foregoing, is the diffusion of the chromatin and the resulting contraction and deeper staining of the nucleus (pyknosis). The appearances clearly indicate that the degenerative process begins at the centre and spreads towards the periphery, and in its course obliterates all the structures of the normal cell (Plate XII, Figs. 3 and 4). After the disruption of the nuclei their fragments may still be retained within the cell body and be further distinguished by marked metachromatic staining (Plate XIII, Fig. 5).

The hyaline transformation does not end the pathological process. Positively chemotactic substances are generated in the areas of cell necrosis, and thereby polymorphonuclear leucocytes are attracted to the areas of dead cells, and not a few of these leucocytes likewise undergo degeneration, often fragmenting and adding their quota to swell the general wreckage. These emigrated cells are found every-

where in the affected parts, in the capillaries, the stroma of the portal spaces, and between and invading the dead cells themselves. They undoubtedly aid in the disintegration of the necrotic masses and the removal of debris. A fibrinoid metamorphosis may affect a part or the whole of the area of dead cells. The outlines of the altered cells, which in all cases are accentuated, now become still more pronounced, and the cell substance about the same time begins to lose its homogeneous and refractive character and to assume a granular and even fatty or vacuolated appearance. Very soon all, or almost all, of the contents, save the much thickened walls (membranes ?) of the cell, disappear. The capillaries having previously been obliterated by the swelling, adjacent rows of cells are brought together, and as the cell contents vanishes, only the coalesced margins of cells are left, thereby producing an appearance like a network of fibrin (Plate XI, Fig. 2).

Hæmorrhages are common in the liver substance, themselves causing more or less damage to the tissue; small extravasations of blood may also take place into the disintegrating necrotic foci. From either cause hepatic cells, quite normal, or hyaline and necrotic, may be washed into the hepatic veins (Plate XIII, Fig. 6).

The blood-vessels of the liver suffer in a more marked degree than those of other organs, except of the local lymphatic glands and the spleen. The changes resemble those found in these organs, the most pronounced alterations being in the spots in which the dead liver cells are contained. Small, light, fibrinous thrombi, almost devoid of cells, may occur in capillaries in regions in which the liver cells are entirely healthy. They are, however, likely to be larger and more common in the areas occupied by dead cells. If attention be directed to the lymphatic spaces in the interlobular connective tissue, one is often surprised at the large amount of nuclear detritus which many of these contain. There can be no reasonable doubt that such an amount of nuclear material could not come from the cells lining the lymphatics, nor from those of the lymph. The only probable interpretation of this appearance is that this material represents largely unclear fragments and debris from the focal areas of necrosis of the liver parenchyma swept along by the lymphatic current.

Kidneys, Adrenal Glands and Heart.—The kidneys showed, in fresh frozen sections, fatty degeneration in some cases only of the tubular epithelium, in others of this and of the glomeruli as well. In very rare cases the glomeruli suffered more than the tubules. The main lesions are to be found in the hardened sections (Flemming's solution, etc.). They occur chiefly in the tubular epithelium, which shows besides the fatty change, hyaline (colloid) transformation and even necrosis, and in the blood-vessels, of which the glomerular capillaries especially are to be mentioned. The endothelium of the glomerular capillaries as a rule did not appear much affected; but within some capillaries leucocytes accumulated, and these rarely underwent fragmentation, or, assuming various bizarre forms, became hyaline, deeply staining, at times fused together, thereby forming imperfect thrombi. The cells of the capillary wall took no part in the process, and it was the rarest occurrence for some material, possibly fibrin, to be deposited upon the capillary walls so as to render them thicker and of a hyaline appearance. The glomerular, but chiefly the capsular epithelium, suffered severely, being degenerated and sometimes converted into ring-shaped bodies (*Schollen* of German writers).

The heart muscle in fully two-thirds of the fatal cases is the seat of fatty degeneration, which may affect almost all or only groups of the fibres. A severer lesion is swelling of the fatty muscle fibres and fragmentation of their nuclei, in other words actual necrosis of the muscle cells.

Hæmorrhages in the adrenal glands, especially marked in the medulla, are invariably present. The cells of the parenchyma and of the vascular endothelial tunic suffer degeneration. The lesions met with here are necrotic, being associated with karyorhexis and affecting, in the former, single cells or small groups. Attention is called to the resemblance of these lesions to those observed in experimental diphtheria.

The thyroid gland also shows much congestion and at times small extravasations of blood.

Brain.—A study of the alterations in the central nervous system

induced by ricin was kindly undertaken, at my suggestion, by Dr. Henry J. Berkley, whose results, with the chrome-silver method, have already been published.* In view of the fact that the silver stain gives only an incomplete picture of the finer lesions of the neurones, it has seemed desirable to supplement this method by others, the best at present being that of Nissl or some of its modifications. This work, which has already been begun, is not sufficiently advanced to warrant a discussion of the results in this paper. Berkley found in animals poisoned with this drug changes especially in the finest nerve processes and involving often wide areas. The alterations consisted of irregular swellings of the dendrites, accompanied by loss of the lateral buds or gemmulæ, followed by complete destruction of the dendrites to the cell body, which itself becomes swollen and more rounded in outline. The cells of the neuroglia, besides swelling and thickening of their extensions, showed no alteration.

Chronic Ricin and Abrin Intoxication.—That individual animals behave in an exceptional manner and succumb after a much longer period of time (6 weeks or longer) has already been pointed out. The pathological picture in such cases is widely different from that described, the extreme emaciation of the animals and the pigmentation of their viscera lending to it the features of a profound cachexia. The microscopical examination bears out this view. Of the acute lesions scarcely an indication can be found. The liver is uniformly dark, and if focal necrosis exists it is in process of organization (one case). Much blood pigment is found in the capillary walls and in cells within their lumina. The spleen shows the most profound changes, which concern not so much its specific elements, but which are to be regarded rather as a result of great hæmolysis. The splenic pulp is crowded with granules and globules of golden yellow pigment which is almost, if not entirely, enclosed in cells either in its sinuses or in its framework and vascular walls. The follicles only are almost devoid of this pigment. Treated with potassium ferrocyanide and hydrochloric

* Berkley, Report in Neurology, iii. *The Johns Hopkins Hospital Reports*, vi, 1896.

acid, all the pigmentary substance strikes a blue color, indicating the presence of an iron constituent more loosely combined than in the hæmoglobin whence it is doubtless derived (hæmosiderin ?). The pigment contained within the liver gives the same reaction. The kidneys may show circumscribed points of granular and fatty degeneration of the epithelium of the labyrinthine tubules, and in addition (one case) a focal increase of cellular connective tissue in the cortex, beneath the capsule, which had given rise to indentations on the surface of the organ.

A comparison of the histological lesions described in the foregoing pages with those found in many forms of bacterial infection and toxæmia demonstrates their essential similarity. The chief lesions, and the ones which are regarded as especially significant and characteristic, are those which are degenerative and focal in character, especially the focal necroses. Inasmuch as similar necroses in the liver and elsewhere play an important rôle, and one which is as yet not generally appreciated, in various human infections and intoxications, the observations recorded in this article are a contribution to our knowledge of the causation and histology of these lesions. As appears from the description, the toxic substances exert their effects upon individual cells, but not all the cells of the body show the same susceptibility to their action. While, as we have seen, lymphoid cells and epithelium are both affected, yet the former are implicated to a greater extent. Neither do all of the epithelial cells of the various organs suffer in equal degree; thus, for example, the cells of the liver are more affected than those of the adrenals and kidneys, and the cells of these latter organs more than those of the pancreas. Finally, in highly differentiated cells, at least, not all parts of the protoplasm need be equally affected by the poison. Not a few examples of partial degeneration of cytoplasm have been described in this article, and if Berkley's results obtained from researches upon the cerebral cortex are confirmed, the constitution of the protoplasm of the dendrites will be proved to be more susceptible than that of the cell bodies.

There is evidence already at hand which goes to prove that the

extent of cell injury in any particular organ is in a measure proportional to the functional activities exerted in dealing with the particular poison. The severity of the intestinal lesions in these forms of intoxication is most probably intimately associated with their eliminative functions.

It is far less easy to account for the focal character of the lesions in the organs, assuming the action to be due to a soluble substance contained within the circulating blood and fluids of the body. While these lesions are produced by the action of soluble toxic substances, their presence cannot be explained by supposing different degrees either of functional activity or of susceptibility to injury in the cells affected. Given an organ such as the liver or the spleen, to which the injurious agents are brought by the circulating blood, we are not justified in assuming either such an irregular function or varying resistance as a consideration of the affected cell groups would, according to this view, render necessary.

I desire to direct attention to another explanation, and one which is more in accordance with observed phenomena, accidental or experimental, than are the foregoing suggestions. I refer to the part played by the circulation of the blood in the affected organs, which I am fain to believe bears an intimate relation to such focally distributed lesions as are now under consideration. We have seen that the action of the poisonous agents is exerted in part upon the capillary walls. The necrosis, fragmentation and regeneration of endothelium indicate this. I would also point out that the injury to the capillary wall is nowhere so great as in the areas in which the necrosis is found. This fact suggests the possibility that in certain capillaries, in which the circulation was much diminished or was temporarily at a standstill at the time when the irritant acted with the greatest intensity, so much damage was done to the vessel wall that a freer transudation than occurred elsewhere took place into the tissues in the neighborhood, resulting in the destruction of cell groups. It is well known that the circulation is not equal in all parts of an organ at all times; this is especially true of the capillary circulation, which is subject to wide variations. Moreover, Cohnheim has shown that the lymph

flowing through a part will be more concentrated the greater the permeability of the vessel wall.

The existence of intracapillary thrombi composed of fibrin and leucocytes in the region of the necrotic cells in the liver was proven by Barker* in his studies upon malaria and by Schmorl† in his work on puerperal eclampsia, and allusion has been made to the occurrence of similar formations in some of my specimens. Both Barker and Schmorl consider it not improbable that these thrombi may have been the precursors of the cell death, an assumption not without pathological foundation. The distribution of the areas of degeneration in lymph gland, spleen, liver, etc., and the frequent involvement of the smallest islands of cells do not, however, agree with the usual findings in anæmic necrosis. Of further significance is the irregular distribution of the thrombi, which occur at one time where no necrosis exists, and at another are absent from considerable areas of cell-death. We have, again, the histological evidence of injury to the vessel walls, which is never absent where parenchymatous necrosis exists, and present even where this other finding is wanting. And if we recall the known relation between degeneration of the vascular endothelium and thrombus formation, it will scarcely be necessary to seek further for an explanation of the observed facts regarding thrombosis.

The uncertainty which must be felt in the study of uninjected specimens as to whether the apparently free capillaries and larger vessels are really open, is removed by examination of the injected organs, a procedure which was carried out in a number of instances of ricin intoxication.

In view of the findings in this study, the recent conclusion of Werhovsky,‡ that in abrin intoxication the essential lesion is swelling of groups of heart muscle fibres, is indeed surprising. He missed entirely the most characteristic and wide-spread lesions.

* Barker, A Study of Some Fatal Cases of Malaria. *The Johns Hopkins Hospital Reports*, v, 1895.

† Schmorl, Pathologisch-anatomische Untersuchungen über Puerperal-Eklampsie. Leipzig, 1893.

‡ Werhovsky, Beiträge zur pathologischen Anatomie der Abrinvergiftung. Ziegler's *Beiträge*, xviii, 115, 1895.

The fate of these lesions in those animals which do not succumb within the period studied, or which entirely recover, is known for at least the liver changes. The invasion of the foci of dead cells by leucocytes and the removal of the necrotic elements may, perhaps, be followed in some instances by regeneration and restoration of the integrity of the organ. I think that it oftener happens that in the place of the dead cells a new tissue develops, which leads not to restoration of the original elements, but to the formation of a scar. The evidence for this rests upon the rich proliferation of tissue cells in capillaries and pre-existent connective tissue about these foci and the gradual invasion of these foci by such cells. While in the series of experimental cases described in the foregoing pages only the acute forms of intoxication have been fully considered, in the study of rabbits poisoned by the blood serum of the dog, in which rabbits similar focal necroses occur, evidence has been found that the areas of necrosis may be replaced by new growths of connective tissue and that thereby a form of cirrhosis may result.

I take pleasure in acknowledging my indebtedness to Dr. William H. Welch for his valuable advice while carrying on these studies in his laboratory.

DESCRIPTION OF PLATES XI–XIV.

PLATE XI.

Fig. 1.—Focal area of necrosis in lymphatic gland (follicle), showing altered cells, fragments of nuclei and metachromatic nuclear particles. Phagocytosis of detritus. Magenta staining. Homogeneous immersion objective $\frac{1}{12}$ in.; eye-piece No. 3.

Fig. 2.—Fibrinoid metamorphosis of liver cells. To the right are liver cells in various stages of hyaline transformation; to the left the fibrinoid change is shown. Remains of liver cell (?) nuclei may be detected among the fibrils. Methylene blue and eosin staining. Objective No. 6; eye-piece No. 3.

PLATE XII.

Fig. 3.—Focal area of necrosis of liver cells and single cell necroses. Karyolysis to be seen in the centre and pyknosis and karyorhexis in the periphery of the nodule. A few fragments of nuclei are also present in the central portion. Methylene blue and eosin staining. Objective No. 3; eye-piece No. 3.

Fig. 4.—A focal area of necrosis similar to Fig. 3. Magenta staining to show the alterations in nuclei and protoplasm. Karyorhexis is more marked than in preceding figure. Same magnification as preceding figure.

PLATE XIII.

Fig. 5.—Necrosis of single liver cell. Swelling of the protoplasm and fragmentation and metachromatic transformation of the nucleus (karyorhexis). The fragments are retained within the cell protoplasm. Safranin staining. Homogeneous immersion objective $\frac{1}{12}$ in.; eye-piece No. 3.

Fig. 6.—Liver cell with fragmented nucleus (or nuclei?) within the central (intralobular) vein of the liver. Magnification and staining the same as in Fig. 5.

Fig. 7.—Spleen of the rabbit, showing foci of nuclear detritus in the Malpighian body, and great dilatation of the blood spaces of the pulp. The latter contain almost no intact cells, but diffusely scattered cell fragments and small agglomerations of blood pigment. Methylene blue and eosin staining. Objective No. 3; eye-piece No. 3.

PLATE XIV.

Fig. 8.—Phagocyte as seen in the spleen and lymphatic glands. In the latter situation the blood pigment is not constantly present. Safranin staining. Homogeneous immersion objective $\frac{1}{12}$ in.; eye-piece No. 3.

Fig. 9.—Macrophage occupying a large venous sinus in the spleen. The contents are degenerated whole cells, fragments of karyo- and cytoplasm and masses of hæmatoidin pigment. Magenta staining. Homogeneous immersion, objective $\frac{1}{12}$ in.; eye-piece No. 3.

Fig. 10.—Intestinal mucosa, showing several crypts of Lieberkühn. Karyorhexis of epithelial elements and slighter fragmentation of the lymphoid cells without. Magenta staining. Homogeneous immersion, objective $\frac{1}{12}$ in.; eye-piece No. 1.

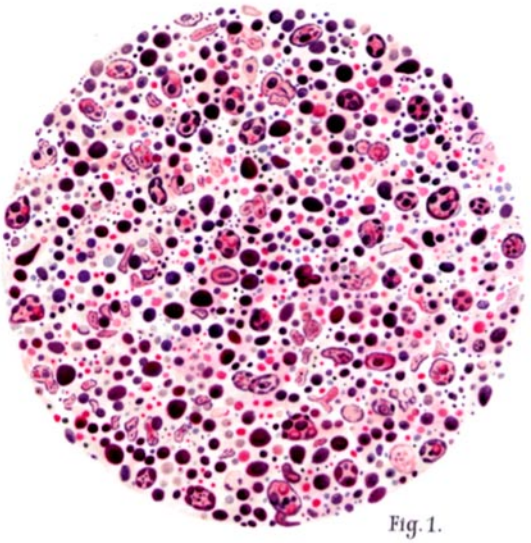

Fig. 1.

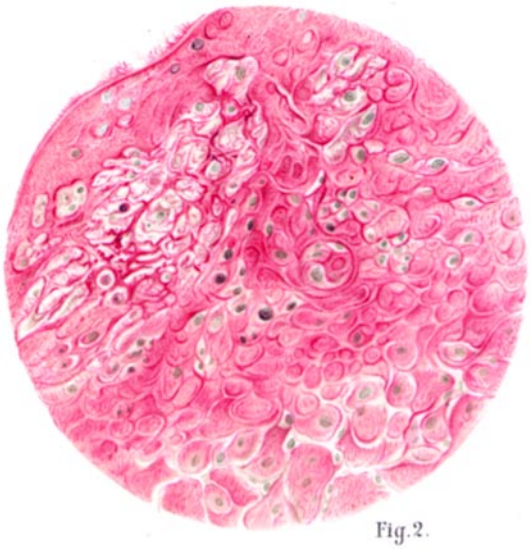

Fig. 2.

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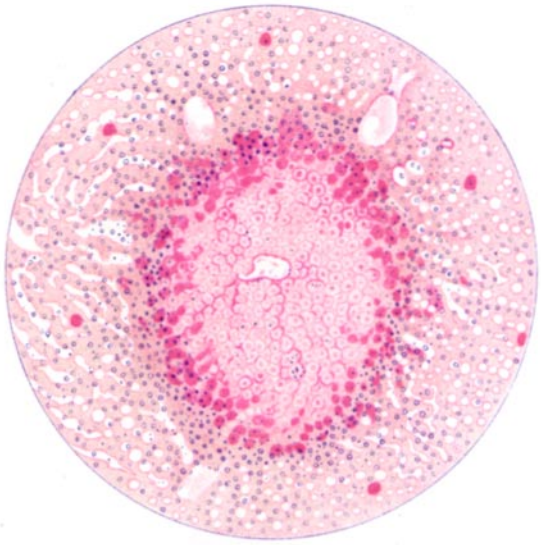

Fig. 3.

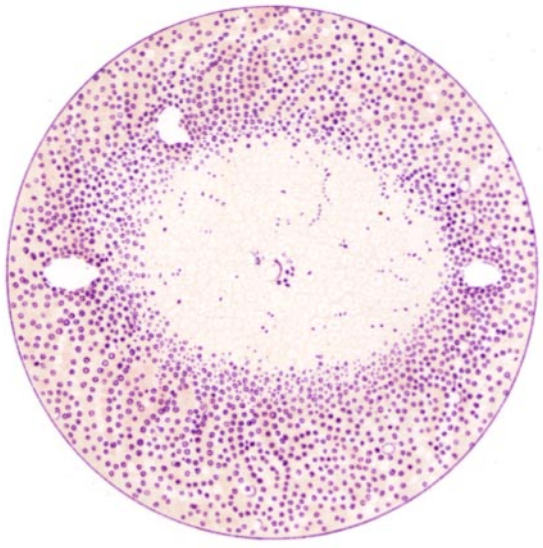

Fig. 4.

Lith. Anst. v. E. A. Funke, Leipzig

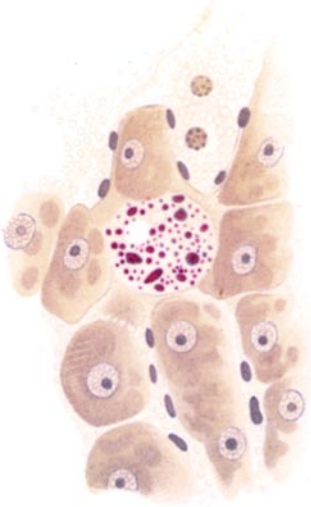

Fig. 5.

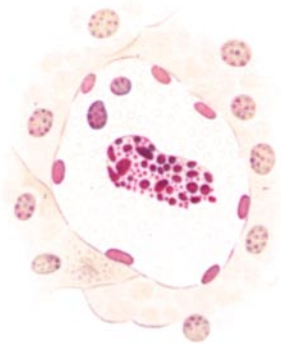

Fig. 6.

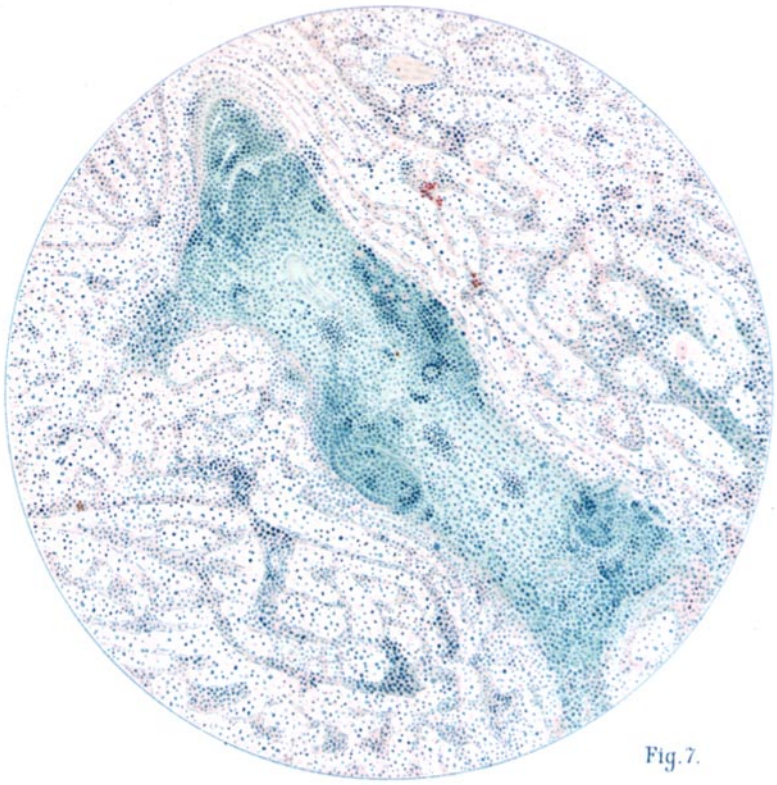

Fig. 7.

Fig. 8.

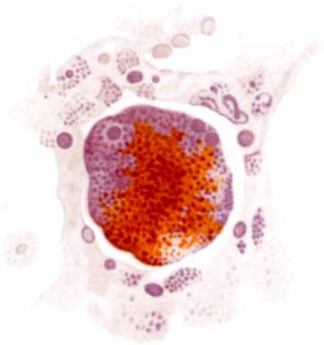

Fig. 9.

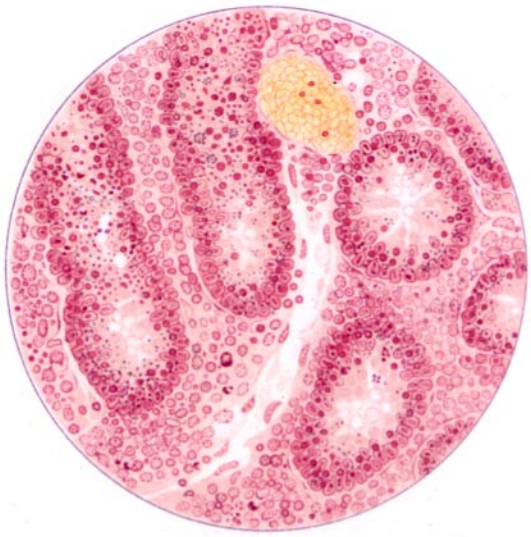

Fig. 10.