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A STUDY OF THE HISTOLOGIC CHANGES PRODUCED EXPERIMENTALLY IN RABBITS BY ARSPHENAMIN *

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The fatalities among persons following the administration of arsphenamin and neo-arsphenamin in the treatment of syphilis, have been mainly attributed to the toxicity of the drugs and to technical errors in administration referable to the preparation of the solution and manner of injection. The results of studies of the gross and histologic tissue changes have usually shown the presence of some form of acute nephritis and degenerative changes in the liver as the most prominent lesions. In Europe an acute hemorrhagic and "serous" meningitis or, rather, an acute meningeal congestion, has been commonly described, whereas in America few or no changes in the membranes and substances of the brain and cord have been found. Under these circumstances, however, only limited information can be gained regarding the tissue changes ascribable to the solutions of the drugs because of pathologic changes in the organs and tissues due to syphilis, for which the majority of persons succumbing after injections of arsphenamin have been treated.

VALUE OF EXPERIMENTAL STUDIES

A study of the effects of arsphenamin and neo-arsphenamin on the tissues of experimental animals has proved of interest in two main

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directions, namely, as bearing on the methods of treatment of syphilis with these compounds and on the subject of specific arsenic therapy.

For example, in reference to the treatment of syphilis, differences in the degree of tissue changes, which were sometimes quite evident, have been found after the intravenous injection of arsphenamin and neo-arsphenamin, and profound differences have been observed according to the kind of solution of arsphenamin administered, as acid and alkaline (disodium) solutions.

In reference to the subject of chemotherapy, we agree with Wade Brown¹ who has well said that the determination of toxicity of new compounds for experimental animals in so far as duration of life is concerned, is insufficient, and that the question of tissue injury has not attracted the attention that the subject deserves.

In comparative study of the changes occurring in the kidneys and suprarenals of experimental animals by various arsenical compounds, Pearce and Brown,² have shown that there may be but slight relation between toxicity and tissue injury and that one may be diminished without necessarily reducing the other; according to their results, it is doubtful whether there is as yet a single compound of arsenic possessing curative value in experimental trypanosomiasis that produces neither toxic symptoms nor organic tissue changes.

The purpose of the experimental chemotherapeutist is, therefore, the production of compounds possessing the maximum of curative value for a certain infection with the minimum of toxicity and tissue injury for experimental animals and persons.

PURPOSES OF INVESTIGATION

The present investigation is a continuation of a series of studies by one of us (Kolmer) with Schamberg and Raiziss,³ bearing on the

1. Brown, W. H.: Lesions Produced by Arsenicals and Their Bearing on the Problem of Specific Arsenic Therapy, *Bull. Johns Hopkins Hosp.* **26**: 309-315, 1915.

2. Pearce, W., and Brown, W. H.: Histological Changes in Arsenic Kidneys, *J. Exper. Med.* **22**:525-534; On the Pathological Action of Arsenicals on the Adrenals, *ibid.* **22**:535-542, 1915.

3. Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: Experimental and Clinical Studies of the Toxicity of Dioxydiamino-Arsenobenzol Dichlorhydrate, *J. Cutan. Dis.* **35**:286 (May-June) 1917. Schamberg, J. F.; Kolmer, J. A.; Raiziss, G. W., and Weiss, C.: Laboratory and Clinical Studies Bearing on the Causes of the Reactions Following Intravenous Injections of Arsphenamin and Neo-Arsphenamin, *Arch. Dermat. & Syph.* **38**:235-256, 1920. Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: Comparative Studies of the Toxicity of Arsphenamin and Neo-Arsphenamin, *Am. J. Med. Sc.* **160**:188, 1920; Comparative Studies of the Trypanocidal Activity of Arsphenamin and Neo-arsphenamin, *ibid.*, to be published.

comparative toxicity and curative activity of arspenamin and neo-arsphenamin from the standpoint of treatment of disease and in relation to experimental chemotherapy with new arsenical compounds being prepared and studied in these laboratories.

In this article are given the results of studies bearing on the histologic changes produced in the tissues of rats and rabbits by different solutions of arspenamin administered in single large and multiple small doses; the results of a similar study with neo-arsphenamin are given separately.⁴

EXPERIMENTAL

White rats and rabbits were employed in all experiments. Arspenamin was administered by injection into the saphenous veins of rats with a gravity apparatus, in the form of 2 per cent. solutions in freshly distilled water at the rate of 0.5 c.c. per minute; rabbits were injected with a syringe by way of the ear veins. Both acid (non-neutralized) and alkaline (disodium) solutions were administered. In preparing the latter the technic of the Hygienic Laboratory was employed, namely, the addition of 0.9 c.c. of a solution of normal sodium hydroxid for each 0.1 gm. of drug; this amount of alkali represents an excess of that required for neutralization and results in a solution of the disodium compound of arspenamin.

Some of the animals were killed at varying intervals after injection and usually by a very brief exposure to illuminating gas which appeared in comparative tests least likely to induce tissue changes, as a number of control animals killed in this manner never exhibited any discernible gross or minute tissue changes. Some animals succumbed at varying intervals after injection; necropsy examinations were performed on these within a few hours after death, and the tissue changes were always identical with those encountered in animals destroyed at chosen intervals by a brief (stupefying) exposure to illuminating gas.

Necropsy examinations were performed immediately after death, and the following organs divided into two parts and placed in Orth's fluid, and in 5 per cent. neutral liquor formaldehydi: cerebrum, cerebellum, brain stem and meninges, lungs, heart, liver, spleen, suprarenals and kidneys. Paraffin and frozen sections were prepared, sections being made of each organ at varying levels; considerable preliminary study was required for the determination of the best technic for the various tissues from the two species of animals. Special studies were made of the suprarenals in reference to the chromaffin

4. Kolmer, J. A., and Lucke, B.: A Study of the Histologic Changes Produced Experimentally in Rabbits by Neo-Arsphenamin, *Arch. Dermat. & Syph.*, this issue, p. 515.

cells and also of the lipid contents, frozen sections being stained with Herzheimer's scharlach r for the latter. Paraffin sections were routinely stained with hematoxylin and eosin.

The tissues of several series of normal rats and rabbits of the same weights and ages as employed in our experiments were studied preliminary to the experimental part in order to have the histologic variations of the different organs under normal conditions for comparative study.

Considerable variation in the degree of tissue change was found among animals of the same species receiving the same drug in the same manner and at the same time; for this reason detailed descriptions may be advisable, but would greatly extend the present article so that a résumé or summary of the changes in the different organs viewed as a whole is here presented.

RESULTS

HISTOLOGIC CHANGES PRODUCED BY THE INTRAVENOUS INJECTION OF ACID SOLUTIONS OF ARSPHENAMIN

When arspenamin is dissolved in water the solution is acid and very toxic for all experimental animals; in previous studies, Schamberg, Kolmer and Raiziss⁵ have determined that failure to neutralize solutions of arspenamin with alkali leads to an increase in toxicity of from 50 to 60 per cent. in solutions of 0.5 to 1 per cent. concentration. A few of the fatalities among human beings have been ascribed to the intravenous injection of acid solutions; in our experiments relatively large doses were employed and all of the animals, excepting one, died within a few minutes after the completion of the injections.

TABLE 1.—THE INTRAVENOUS INJECTION OF ACID SOLUTION OF ARSPHENAMIN IN RATS

Weight, Gm.	Sex	Dose per Kilo	2 Per Cent. Solution, C.c.	Results
105	F	0.100	0.53	Immediate death
140	M	0.090	0.63	Immediate death
85	M	0.080	0.34	Killed 12 hours later
95	M	0.070	0.33	Immediate death
130	M	0.060	0.39	Immediate death
110	M	0.050	0.28	Immediate death

Table 1 gives the results observed after the intravenous injection of acid solutions of arspenamin in rats and Table 2, of similar injections in rabbits; the tables show the doses employed.

5. Schamberg, J. F.; Kolmer, J. A., and Raiziss, G. W.: Experimental and Clinical Studies of the Toxicity of Dioxydiamino-Arsenobenzol Dichlorhydrate, J. Cutan. Dis. **35**:286 (May-June) 1917.

TABLE 2.—THE INTRAVENOUS INJECTION OF ACID SOLUTION OF ARSPHENAMIN IN RABBITS

Weight, Gm.	Dose per Kilo	2 Per Cent. Solution, C.c.	Results
1,580	0.030	2.4	Died at once
2,660	0.040	5.3	Died at once
1,650	0.050	4.1	Died at once
2,450	0.060	7.4	Died at once
1,740	0.080	7.0	Died at once

The principal tissue changes were:

Cerebrum, Cerebellum, Brain Stem and Meninges.—In both rats and rabbits hyperemia of the meninges, and especially of the pia-arachnoid, was found; in many of these vessels were conglutinated or even hyalinized masses of erythrocytes in which the outlines of the individual cells were poorly defined or entirely lost. Occasional small hemorrhagic areas were found in different parts of the brain and in the meninges. Thrombi of conglutinated erythrocytes were also found in some of the smaller vessels of the cerebrum and cerebellum, but there were no discernible changes in the ganglion cells.

Lungs.—Vascular changes were most prominent. The majority of the blood vessels contained thrombotic masses, often possessing a light yellowish green color and in the larger vessels presenting a peculiar whorl-like appearance (Figs. 1, 2, 3, 4). The bands of these thrombi were usually homogeneous, but were sometimes granular; individual erythrocytes were only occasionally to be discerned. The capillaries of the alveolar walls in some areas were engorged with conglutinated erythrocytes; in other areas they were quite empty and even collapsed. Hemorrhage into the air sacs and interstitial tissue was found in only a few scattered areas; no other changes of importance were present.

Heart.—The most prominent changes were distention of the right auricle and ventricle with clots composed of more or less conglutinated erythrocytes; embedded in these clots were found the same peculiar yellow-green tinted whorl-like bands of homogeneous material seen in the larger pulmonary vessels (Fig. 5). These "acid-arsphenamin thrombi" were different in morphology and staining from the ordinary "hyalin thrombi" frequently found in various toxic and infectious diseases. The capillaries of the heart wall were generally intensely congested, and many contained conglutinated or hyalinized masses of erythrocytes. In a few instances rupture of capillaries with resulting small focal hemorrhages between the muscle bundles were encountered. Here and there anemic areas were found, apparently due to vascular occlusions.

Liver.—Intense hyperemia and widespread but irregularly distributed vascular occlusion, with conglomerated and partly or entirely hyalinized masses of erythrocytes, characterized the most conspicuous alterations (Fig. 6). In view of the tissue changes found in many animals of the other experimental series, this irregular thrombosis is to be emphasized. Most often the central lobular veins and the capillaries around these vessels were prominently distended, either with fairly well preserved erythrocytes, or, more frequently, with red cells more

Fig. 1.—Lung from rabbit injected with acid arsphenamin; immediate death. All the larger vessels are occluded by thrombi, details of which are shown in Figures 2, 3 and 4. (Very low magnification.)

or less fused or even hyalinized. Sometimes the peripheral vessels (the branches of the hepatic artery and portal vein and the peripheral capillaries) were similarly distended, while the central lobular veins and the adjacent capillaries were almost empty. No changes in the liver cells were discernible when death followed immediately after the injection, but in the one animal which survived five hours, early necrotic changes were observed in numerous irregularly distributed areas but

particularly around the intralobular veins. These areas gave the impression of early infarctions and were sometimes flooded with escaped erythrocytes.

Kidneys.—Marked congestion and large numbers of thrombi of conglutinated erythrocytes mainly in the vessels of the boundary zone, constituted the predominating change; the malpighian tufts were usually greatly engorged and generally filled the capsular space. Many of the component capillaries contained hyalinized or conglutinated erythrocytes, while others were without contents and collapsed. Hemorrhages were

Fig. 2.—Large pulmonary artery from rabbit injected with acid arsphenamin; immediate death. Occlusion with a peculiar type of thrombus which consists of light yellow-green colored homogeneous bands arranged in a whorl-like formation ("acid-arsphenamin thrombus"). (High dry lens magnification.)

not found; changes in the tubular epithelium were not observed except in the one animal that survived five hours, marked cloudy swelling and coarse granularity of the cells of the convoluted tubules especially being encountered.

Suprarenals.—The only prominent and constant change was well marked congestion of the medullary portion, with numerous small thrombi similar to those found in the other organs; there were no discernible changes in the lipoid and chromaffin contents.

Spleen.—This organ usually showed an intense congestion and numerous thrombi of conglomerated erythrocytes; the follicles and trabecula presented no changes.

HISTOLOGIC CHANGES PRODUCED BY THE INTRAVENOUS
INJECTION OF SINGLE LARGE DOSES OF SOLU-
TIONS OF DISODIUM ARSPHENAMIN

In the routine toxicity tests of arsphenamin white rats weighing from 100 to 150 gm. are injected with 2 per cent. solutions of disodium arsphenamin in doses equivalent to 0.1000 gm. per kilogram of body

Fig. 3.—Large pulmonary artery and part of veins from rabbit injected with acid arsphenamin; immediate death. Both vessels are occluded with thrombi of the type shown in Figure 2. (High dry lens magnification.)

weight; this amount is equivalent to 6 gm. per 60 kilos of body weight. The tissues of rats succumbing at varying intervals after these injections, as shown in Table 3, have been examined as part of our study.

As a general rule, rabbits are somewhat more susceptible to the toxicity of arsphenamin. A number were given intravenous injections of 0.080 gm. of disodium arsphenamin per kilo of body weight, corresponding to 4.8 gm. per 60 kilos, and examined at varying intervals after injection, as shown in Table 4.

In some instances the animals had been dead about twelve hours before necropsy examinations were performed; however, the majority of animals were killed at intervals and the necropsy examinations were performed within an hour.

TABLE 3.—THE INTRAVENOUS INJECTION OF RATS WITH SINGLE LARGE DOSES OF SOLUTION OF DISODIUM ARSPHENAMIN

Weight, Gm.	Sex	Dose per Kilo	Results
100	M	0.100	Lived 2 days
125	M	0.100	Lived 10 days
100	M	0.100	Lived 11 days
110	M	0.100	Lived 12 days
150	M	0.100	Lived 13 days

TABLE 4.—THE INTRAVENOUS INJECTION OF RABBITS WITH SINGLE LARGE DOSES OF SOLUTIONS OF DISODIUM ARSPHENAMIN

Weight, Gm.	Dose per Kilo in 10 C.c.	Results
1,690	0.080	Examined 3 days after injection
2,230	0.080	Examined 5 days after injection
1,640	0.080	Examined 7 days after injection
2,150	0.080	Examined 12 days after injection

The histologic changes may be summarized as follows; marked differences were found according to the duration of life following the injections:

Cerebrum, Cerebellum, Brain Stem and Meninges.—Sections of different portions of the cerebrum and cerebellum have shown no striking or constant changes. The brain vessels were moderately engorged in some animals; here and there clear zones of perivascular edema were found. Occasionally a few isolated degenerated ganglion cells were encountered. The meninges have generally shown well marked congestion and a few hyalinized thrombi. Slight meningeal edema and occasional small round cells were often encountered, but leukocytic infiltration was not seen in any section. In two animals surviving for more than ten days several minute hemorrhages were found in the brain substance.

Lungs.—Vascular changes were most prominent but varied according to the duration of life after the injection of arspenamin; these changes were also somewhat more marked among the rats than among the rabbits.

When the lungs were examined within two days after injection of arspenamin, marked congestion and small thrombi of conglutinated erythrocytes were seen associated with scattered areas of edema and extravasation of erythrocytes into the air sacs. After longer intervals

between injection and death less marked congestion and edema were usually found. Catarrhal swelling and inconspicuous desquamation of the lining alveolar epithelium was frequently encountered. Thrombosis was practically never seen. The terminal bronchi and bronchioles frequently exhibited slight mucopurulent inflammatory changes; the latter, however, may not have been caused by the drug.

Heart.—No constant and striking changes were found; the epicardium and endocardium appeared normal, but in a few animals, and especially among the rats that died within a few days after injection,

Fig. 4.—Large pulmonary artery and veins from rabbit injected with acid arsphenamin; immediate death. The erythrocytes are partly conglutinated and in places fused into hyaline masses. Thrombi of this type occur frequently with the peculiar "acid arsphenamin thrombi" shown in Figures 2 and 3. In Figure 1 "conglutination," "hyaline" and "acid arsphenamin thrombi" are shown. (Low dry lens magnification.)

small areas of focal parenchymatous degeneration were encountered in the myocardium. In these areas the muscle cells appeared cloudy, swollen, hyalinized, vacuolated, devoid of striations, and presented nuclear degeneration. In some instances the smaller vessels were congested and occasionally plugged with thrombi of conglutinated erythrocytes, which were probably responsible for the areas of focal degeneration. In several animals rather diffuse cloudy swelling of the

Fig. 5.—Clot in right ventricle from rabbit injected with acid solution of arsphenamin; immediate death. Embedded in the mass of partly conglutinated erythrocytes are the yellow-green colored, homogeneous bands of the "acid arsphenamin thrombi." (Low dry lens magnification.)

Fig. 6.—Liver from rat injected with acid arsphenamin; immediate death. Extreme congestion and occlusion of the central lobular veins and intralobular sinusoids with conglutinated and partly hyalinized erythrocytes. The blood channels of the organ stand out as boldly as if they were injected with a coloring mass. (Low dry lens magnification.)

muscle fibers were seen; slight swelling of the vascular endothelium sometimes occurred. In one rat, succumbing after ten days, an area of necrosis with well marked infiltration by small round cells and fewer polymorphonuclear leukocytes was present.

Liver.—The liver has shown extensive and striking changes in all animals, but particularly among the rats; these changes were mainly in the form of extensive areas of coagulation necrosis involving all parts of the lobule but particularly the central and middle portions.

Fig. 7.—Liver from rat injected with a single large dose of disodium arsenamin; death two days after injection. There is a massive necrosis involving all but the lobular peripheries. (Low dry lens magnification.)

Rats succumbing within two days after the injection have shown changes so extensive as to resemble those commonly found in acute yellow atrophy, entire lobules, excepting the most peripheral portions, being necrotic and sometimes flooded with erythrocytes and infiltrated with mononuclear and fewer polynuclear leukocytes (Fig. 7). Among animals surviving a week or longer, the areas of necrosis were not so

large but were found in various parts of the lobule including the peripheral portions (Figs. 8 and 9). The irregular distribution of these necrotic areas is noteworthy.

The different stages of the necrotic process could be well recognized. The earliest changes occurring in animals that survived only a day or two were extensive coagulation necrosis, probably due to infarction. Later complete breaking down of the necrotic portions occurred, which then became flooded with erythrocytes and invaded by various phago-

Fig. 8.—Liver from rat injected with a single large dose of disodium arspenamin; death ten days after injection. Many large irregularly distributed areas of necrosis are present, some are partly flooded with erythrocytes. (Low dry lens magnification.)

cytic cells. The bile ducts usually showed no changes other than occasionally swollen epithelium. Many of the animals presented portal, as well as intralobular, congestion. Thrombi of conglutinated and hyalinized corpuscles were frequently seen in the vessels of the capsule, within the lobules and in the necrotic areas. Inconspicuous perilobular fibrosis was encountered only among animals surviving ten days or longer.

Kidneys.—Marked degenerative changes were generally found in all animals, but the kind and severity of the changes varied according to the duration of life following the injection of arsphenamin; as a general rule, the kidneys of rats showed somewhat greater changes than those of rabbits, although this may be expected by reason of the larger doses administered to the former.

Among animals succumbing within two days after injection, vascular changes predominated; among those living for longer intervals,

Fig. 9.—Liver from rat injected with a single large dose of disodium arsphenamin; death eleven days after injection. Many areas of irregularly distributed necrosis are present. (Low dry lens magnification.)

tubular degeneration was more prominent with marked variations in the severity and extent of the lesions.

The vascular changes consisted principally of marked congestion of the vessels of the boundary zone and cortex; occasionally minute interstitial hemorrhages were seen. Numerous small thrombi of coagulated erythrocytes were encountered. The glomerular tufts were generally greatly engorged and in part occluded with hyalinized

thrombi; some of the loops were collapsed. The capsules of Bowman usually showed no changes of the lining epithelium, but some fluid and granular debris were frequently found in the intracapsular space. Even in the animals dying after a short period, pronounced degeneration of tubular epithelium occurred, but the parenchymatous changes were generally greatest in animals surviving for a week or longer. The mildest changes consisted of extensive cloudy swelling, vacuolization and granularity, involving particularly the convoluted tubules of the inner cortical zone. While the limbs and loops of Henle sometimes showed considerable cellular degeneration, this change was never as pronounced as in the convoluted tubules. The collecting tubules were least affected.

Fig. 10.—Kidney from rabbit injected with a single large dose of disodium arsphenamin; death ten days after injection. Large patches of the cortex are entirely necrotic and heavily infiltrated with calcium salts (which take a black tint in the photograph). The medullary portions of the kidneys were never necrotic. (Very low power magnification.)

The degenerative alterations were of all degrees; several animals surviving about five to seven days showing almost complete necrosis of the outer half of the cortex. The necrotic regions were heavily infiltrated with lime salts (Figs. 10, 11 and 12).

A curiously irregular distribution of the tubular degeneration and necrosis was noted. Thus, often only isolated tubules were necrotic

while the neighboring tissue was in a fair state of preservation. In many instances the distribution was such as to point to infarction (Figs. 13 and 14).

In the extreme necrosis all elements of the renal tissue in the involved areas were completely destroyed.

Granular debris, erythrocytes, and desquamated cells frequently formed tubular casts.

Fig. 11.—Kidney from a rabbit injected with a single large dose of disodium arsphenamin; death eleven days after injection. Great necrosis of cortical regions, which are heavily infiltrated with calcium salts (black in the photograph). In an attempt to bring out the necrotic portions some of the intervening tissue is thrown out of the proper focus. (Low dry lens magnification.)

Slight edema was often found. The various changes pointed strongly to individual difference in susceptibility or resistance among the animals.

Among the kidneys of animals surviving about two weeks, slight interstitial fibrosis was evident in both cortex and medulla, and especially about areas of marked tubular necrosis.

Suprarenals.—In practically all animals there was great alteration in the lipoid and chromaffin contents.

Among the animals surviving about two days the lipid material was present only in the inner border of the zona fasciculata and occurred chiefly in the form of large droplets. In the zona glomerulosa the cells were almost entirely free of lipid substances. Among the animals surviving for ten days or longer the lipid material was likewise changed from finer granules to coarser droplets and showed evidences of depletion, being principally limited to the outer or inner border of the zona fasciculata.

The chromaffin contents of the medullary cells was diminished, more especially in the animals surviving for five days or longer, indi-

Fig. 12.—Kidney from rabbit injected with a single large dose of disodium arspenamin; death eleven days after injection. A portion of the renal medulla is shown; many collecting tubules contain casts composed chiefly of conglutinated erythrocytes and necrotic renal epithelium. (Low dry lens magnification.)

cating a gradual rather than sudden exhaustion, although this change was not constant.

Many of the cells in both medulla and cortex showed cloudy swelling and nuclear degeneration.

Many of the suprarenals showed moderate congestion of the medulla and cortex, particularly of the former, and a few small thrombi of conglutinated erythrocytes.

Interstitial changes, such as cellular infiltrations and fibrosis, were not encountered.

Spleen.—These organs frequently presented striking irregular areas of coagulation necroses, especially in those removed from the rats.

Among the animals succumbing within two days of injection, decided congestion of the splenic pulp and numerous small thrombi of coagulated erythrocytes in the vessels and sinuses were commonly found. At this time, more especially among animals surviving for longer periods, the pulp cords showed marked necrosis with some polymorphonuclear leukocytic infiltration and many megalokaryocytes.

Fig. 13.—Kidney from rabbit injected with a single large dose of disodium arsphenamin; death eleven days after injection. Several large cortical patches are in a stage of partial necrosis, probably due to incomplete vascular occlusion (incomplete infarction). (Low dry lens magnification.)

These areas of coagulation necrosis were usually focal, small and distributed, but in some organs were quite large and had an almost hyaline and homogeneous appearance. Unusually large numbers of broken-down erythrocytes were seen; a fine hemosiderin pigmentation was found.

As a general rule, the follicles were fairly well preserved and there was apparently no involvement of the trabecula and capsule.

HISTOLOGIC CHANGES PRODUCED BY INTRAVENOUS INJECTIONS
OF MULTIPLE SMALL THERAPEUTIC DOSES OF SOLUTIONS
OF DISODIUM ARSPHENAMIN

When a solution of arspenamin is neutralized with sufficient sodium hydroxid to just dissolve the precipitate and about one third more of this amount of alkali is added, a disodium compound results which is believed by some to be somewhat less toxic than solutions to which just enough alkali is added to dissolve the precipitate and "clear" the solution.

TABLE 5.—THE INTRAVENOUS INJECTION OF RATS WITH A SOLUTION OF DISODIUM ARSPHENAMIN EVERY THREE DAYS

Weight, Gm.	Sex	Dose per Kilo	2 Per Cent. Solution, C.c.	Number of Injections
150	M	0.010	0.075	10
85	M	0.010	0.043	8
120	M	0.010	0.06	10
130	M	0.010	0.065	10
140	M	0.010	0.07	10
140	M	0.010	0.07	10

TABLE 6.—THE INTRAVENOUS INJECTION OF RABBITS WITH MULTIPLE DOSES OF A SOLUTION OF DISODIUM ARSPHENAMIN

Dose per kilo, 0.01 c.c.

	Weights on Day of Injection					
January 27.....	1,670	2,050	1,730	1,680	1,970	1,640
30.....	1,635	2,040	1,640	1,575	1,985	1,495
February 2.....	1,390	2,100	1,680	1,500	2,000	1,330
5.....	1,380	2,040	1,640	1,440	1,880	1,430
9.....	1,390	1,970	1,570	1,460	1,880	1,430
12.....	1,470	2,050	1,695	1,490	1,970	1,480
16.....	Exam.	Exam.	1,575	1,430	1,830	1,965
19.....			1,700	1,575	1,940	2,090
24.....			1,540	1,545	1,840	1,850
26.....			1,645	1,560	1,830	1,895
March 1.....			1,640	1,520	1,875	1,660
4.....			1,565	1,470	1,865	1,680
8.....			Exam.	Exam.	1,830	1,580
11.....					2,000	1,480
15.....					1,960	1,550
18.....					1,910	1,640
22.....					1,920	1,635
					Exam.	Exam.

A series of six rats were given from eight to ten intravenous injections of solutions of the disodium compound of arspenamin in doses of 0.01 gm. per kilogram of weight, corresponding to 0.6 gm. per 60 kilograms, which is the maximum dose usually administered at one time to human beings in the treatment of syphilis (Table 5); these animals were killed and necropsy examinations made about twenty-four hours after the last injection. The injections were given twice a week.

A series of six rabbits were given from six to eighteen injections of solutions of the disodium compound in the same amounts, namely, 0.01 gm. per kilo corresponding to 0.6 gm. per 60 kilos of weight, twice a week; animals were killed and necropsy examinations made after six, twelve and eighteen doses, as shown in Table 6.

The histologic examination of these tissues has proved of considerable interest, as the drug was administered in multiple small doses in a manner analogous to its administration to persons.

Fig. 14.—Kidney from rabbit injected with a single large dose of disodium arsphenamin (from rabbit whose kidney is shown in Figure 13). A portion of the cortex shows great tubular dilatation, tubular collapse, atrophy and degeneration of the lining epithelium. Many casts are present. (High dry lens magnification.)

Cerebrum, Cerebellum, Brain Stem and Meninges.—Inconspicuous changes were found. The meninges have generally shown a slight degree of hyperemia, but no cellular infiltrations; thrombi of conglutinated erythrocytes were generally absent, although a few such were found. Occasionally extravasated red cells were found. The vessels of the brain were slightly congested, and in some specimens slight perivascular edema was noted. The vascular endothelium sometimes appeared swollen. In sections of the brains of some of the rats, num-

erous irregularly distributed minute areas of focal hemorrhage were seen (Fig. 15), a few being surrounded by definite zones of early necrosis in which the cells were stained diffusely and indistinctly and the details of morphology obscured. These changes occurred particularly in animals that had received many injections.

Lungs.—The lungs of both series of animals have shown interesting and instructive changes. In specimens of animals receiving the maximum number of injections, the capillaries were tortuous and densely packed with erythrocytes, which sometimes were conglutinated. The walls of the air sacs were somewhat thickened and more cellular, due

Fig. 15.—Brain from rat injected with ten small doses of disodium arspenamin. A small focal hemorrhage is shown. (Low dry lens magnification.)

to proliferating fibrous tissue. In large areas the alveoli were filled with edematous fluid, in which hemosiderin-carrying phagocytes could here and there be seen. The whole picture suggested passive congestion of moderately long standing with diffuse capillary edema. In the animals receiving fewer injections, these alterations were less noticeable.

A few of the larger pulmonary vessels (of rabbits) contained partly conglutinated or hyalinized thrombi. In one animal which received six

injections a large vein was completely occluded by closely interwoven hyaline bands of whorl-like arrangement; calcium granules were scattered throughout this thrombotic mass. In another rabbit which had received the maximum number of injections an interesting thrombosis occluded four fifths of the large vein. This thrombus was in a stage of advanced organization, being composed of young connective tissue cells, between which was found a heavy deposit of calcium. A portion of this calcium was present in the form of coarse granules which were surrounded by syncytial giant cells; elsewhere the lime salts occurred in dense clumps. A new channel had formed lined by endothelium and containing well preserved red cells (Fig. 17).

Fig. 16.—Lung from rat injected with ten small doses of disodium arsphenamin; an area of bronchopneumonia with adjacent compensatory inflated lung tissue. A medium sized artery presents considerable thickening of its wall and narrowing of the lumen. (Low dry lens magnification.)

These thrombi probably illustrate a not infrequently occurring condition, namely, arsphenamin, even in small doses may occasion a few pulmonary clots, which, while not interfering with life, may produce some symptoms.

Some of the arteries exhibited slight proliferative endarteritis with swollen endothelium and proliferation of the subendothelial connective tissue, but in this connection it is well to remember that rabbits are prone to develop spontaneous arteriosclerosis.

The lungs of the rats exhibited the same kind of vascular changes but in a lesser degree; patches of bronchopneumonia were frequently found (Fig. 16). In these areas the bronchial walls were generally infiltrated with polymorphonuclear cells and the lumen filled with masses of degenerating leukocytes and cellular débris. The surrounding air sacs were almost entirely filled with leukocytes, a few erythro-

Fig. 17.—Large pulmonary vein from rabbit injected with seventeen small doses of disodium arsphenamin. An organized thrombus fills about four-fifths of the lumen. A heavy calcium salt deposit is shown. A new channel relined with endothelium has formed. (High dry lens magnification.)

cytes and some desquamated epithelium or mixtures of edematous fluid and cells. In these areas of pneumonia, the alveolar walls were sometimes necrotic and infiltrated with polymorphonuclear leukocytes. In this connection it may be stated that both rats and rabbits, and par-

ticularly the former, are very susceptible to pulmonary infection. The animals having received the largest number of injections were particularly prone to exhibit inflammatory alterations.

In other areas free of pneumonia, the alveolar walls were prominent, their capillaries tortuous, often engorged and sometimes occluded by conglomerated red cells. Fibrous thickening of the alveolar walls, in a moderate degree, was found when the animals had received many injections.

The pleura showed no changes.

Fig. 18.—Heart from rat injected with ten small doses of disodium arsenophenamin. In a large part of the myocardium the muscle fibers are degenerated; between these fibers a fairly dense round cell infiltration exists. The degenerative and cellular infiltration appear sharply limited. (Very low magnification.)

Heart.—Sections of the hearts of some rats showed irregularly distributed focal areas of degeneration situated sometimes near the endocardium, sometimes beneath the epicardium or deep in the myocardium. The muscle degeneration in these areas was of various types, showing cloudy swollen fibers with indistinct or absent striations, homogeneous and hyalinized protoplasm and considerable vacuolization. Many of the muscle cells were mere broken-up structureless atrophic masses. In

these areas of focal necrosis were dense collections of large and small mononuclear cells with some fibroblasts and a few plasma cells; polymorphonuclear elements were inconspicuous. These areas of necrosis were evidently of different ages, as if some muscle degeneration had just begun, whereas in others degeneration was advanced and cellular infiltration quite extensive. Much variation as to size was present, but generally the areas of focal necrosis were quite small. In several instances they were so sharply limited as to suggest that only definite bundles were involved. This seemed to point toward infarction, or at least local anemia. Generally, the degree of tissue alteration stood in proportion to the medication received (Figs. 18 and 19).

Fig. 19.—Heart from rabbit injected with ten small doses of disodium arspenamin. This is a high magnification of a field shown in Figure 18. Between the degenerated muscle cells are large numbers of infiltrating small and large round cells. Polymorphonuclear leukocytes are rarely encountered in such areas.

The hearts of the rabbits also showed occasionally slight focal necrosis, but the changes were not so striking as in the hearts of the rats. Slight passive congestion, inconspicuous swelling of vascular endothelium, occasional small hyalinized thrombi and slight interstitial edema constituted the usual changes.

Liver.—Slight periportal fibrosis with small areas of focal necrosis, particularly in the peripheral portions of the lobules, constituted the most conspicuous changes in this organ.

The fibrosis was confined almost entirely to tissue about the blood vessels and bile ducts, and was never sufficiently extensive to surround a lobule.

The vascular changes were never great; a few thrombi of partly hyalinized masses of erythrocytes were found and only occasionally passive congestion of the intralobular veins. No changes were found in the bile ducts.

The areas of focal necrosis were found more especially in the rat's organs; these were quite small and generally in the peripheral portions

Fig. 20.—Spleen from rat injected with six small doses of disodium arsenamin. Many clumps of hemosiderin (black in photograph) are scattered throughout the tissue. In animals receiving the maximum number of injections the hemosiderin pigmentation is considerably more pronounced. (Low dry lens magnification.)

of the lobules, although this distribution was frequently very irregular. The appearance of these areas varied from groups of necrotic cells sometimes flooded with erythrocytes or areas of marked necrosis infiltrated with lymphocytes, to definitely fibrous round nodules in animals that had received the largest number of injections. The connective tissue proliferations were only discernible in the organs of those animals receiving the maximum number of injections.

Kidneys.—Vascular changes constituted the most prominent alterations embracing marked congestion, especially pronounced in the deeper cortical and outer boundary zones. The dilated vessels frequently contained thrombi of partially or entirely hyalinized masses of erythrocytes. The glomeruli, corpuscles and capsular spaces showed no abnormal changes, except that the vessels of the tufts were generally distended and frequently occluded with the hyalinized plugs of erythrocytes; collapsed glomerular capillaries were sometimes seen. Occasionally minute interstitial hemorrhages were found, likewise slight degrees of interstitial edema.

The tubules have usually shown minor changes generally confined to the convoluted tubules of the outer cortical zone and spiral portions of the outer boundary zone; the collecting tubules have shown no changes. In the involved areas the tubular cells have been found cloudy, swollen with granular and vacuolated protoplasm and degenerated nuclei. Here and there an inconspicuous connective tissue increase was encountered. This was seen only in animals receiving the maximum number of injections, and generally consisted of small groups of spindle cells or small round cells, generally situated about a glomerulus. Frank necrosis was not encountered. Evidences of cellular regeneration were frequently seen.

Suprarenals.—These organs have shown considerable variation among animals receiving the same amounts of arsphenamin per body weight at the same time and examined by necropsy at the same intervals after injection. In all instances the suprarenals were removed and fixed within an hour after death.

In the animals receiving the smaller number of injections a definite increase in size and quantity of the cortical lipoid was observed. All three zones of the cortex exhibited this lipoid increase, but the middle zone was the seat of the greatest accumulation. In the animals receiving the largest number of injections a slight lipoid exhaustion was present, particularly affecting the outer zone.

There appeared to be a distinct swelling of the cortical cells, and frequently many cells were seemingly regenerating.

The vascular changes were slight, the capillaries being occasionally engorged with conglutinated or hyalinized masses of red cells. The larger vessels showed no alterations. There was no change in the interstitial substance.

In the medullary portions many of the cells were shrunken, and while most contained some chromaffin, there were many that did not, and we are of the opinion that the majority of sections showed slight chromaffin depletion.

Spleen.—The principal change was one of passive congestion with slight hemosiderosis and fibrillar fibrosis, particularly in the animals receiving the maximum number of injections (Fig. 20). The capsule and trabeculae showed no structural changes, or at most slight fibrosis in those animals receiving ten or more injections. A few giant cells resembling megalokaryocytes were occasionally encountered, and the malpighian follicles were generally rather large, with prominent germinal centers.

SUMMARY AND DISCUSSION

It is to be emphasized that considerable individual variations in the histopathologic changes were found in the various organs of animals receiving the same drug under similar experimental conditions; general changes may be summarized as follows:

1. *Changes Produced by Acid Solutions of Arsphenamin.*—1. The blood vessels of the organ examined (brain and meninges, lungs, heart, liver, spleen, kidney and suprarenal) were either intensely engorged or occluded by thrombi consisting of partly or entirely conglutinated or hyalinized erythrocytes.

2. This thrombosis was irregularly distributed.

3. In the larger pulmonary vessels and in the distended chambers of the right side of the heart the thrombi were a peculiar light yellow-green, and consisted of homogeneous bands arranged in whorl-like formation ("acid-arsphenamin thrombi").

4. Occasional small capillary hemorrhages were found.

5. There were no tissue alterations (death having occurred immediately after injection), excepting in one animal which survived for five hours and which exhibited early parenchymatous degeneration, particularly in the liver and kidney.

2. *Changes Produced by Single Large Doses of Solutions of Disodium Arsphenamin (0.100 gm. per kilo or 6 gm. per 60 kilos).*—1. The tissue alterations were prominent and depended chiefly on the number of days the animal survived the injection.

2. Degenerative tissue changes predominated over the vascular changes. In the liver unevenly distributed areas of necrosis, often of extreme extent, were seen. The kidneys exhibited extensive tubular necrosis and calcium deposition; the spleen often presented irregular areas of coagulation necrosis; in the suprarenals there was a depletion of the lipid and chromaffin contents. Areas of focal necrosis were found in the myocardium. The brain tissue was little altered.

3. The vascular changes consisted of more or less pronounced congestion and irregularly distributed conglutination and hyaline thrombi;

these may be in part responsible for the degenerative changes. Small focal hemorrhages were frequently found.

4. These changes were produced by arspnenamin prepared by three different laboratories.

3. *Changes Produced by Multiple Small Doses of Solution of Disodium Arspnenamin (0.01 gm. per kilo or 0.6 gm. per 60 kilos).*—1. The lesions in these series were inconspicuous and varied according to individual susceptibility and the number of doses injected.

2. A very slight connective tissue proliferation was found in the lung, liver, spleen and kidney of the animals receiving the maximum number of injections.

3. More or less marked chronic passive congestion was found with moderate hemosiderosis in the spleen; inconspicuous amounts of hemosiderin occurred in the lung.

4. Occasional vessels contained thrombi composed of partly or entirely conglutinated or hyalinized erythrocytes. In the lung of two animals organized thrombi were found.

5. Areas of bronchopneumonia, of doubtful origin, were encountered.

6. Areas of focal necrosis, inconspicuous in the heart and more pronounced in the liver, were seen.

7. The lipoids of the suprarenals were at first increased in quantity; later a slight exhaustion appeared. An ill-defined chromaffin exhaustion took place.

8. Parenchymatous changes of mild degree were seen, but these were never of such degree as to interfere with the function of the various organs.

9. These changes were produced by arspnenamin prepared by three different laboratories.

In general it would appear that the primary changes produced by arspnenamin are vascular in nature and consist of more or less marked congestion and the production of numerous thrombi by conglutination of erythrocytes, which later may become more or less hyalinized.

These vascular phenomena have been found in varying degree in all of the organs examined and have been most severe after the intravenous injection of acid solutions of arspnenamin, and much less evident after the administration of the alkaline or disodium solution.

The constitution of the thrombi are especially interesting in view of the theory of Danysz,⁶ that carbon dioxid and sodium biocarbonate

6. Danysz, W.: Ann. d. l'Inst. Pasteur **31**:114, 1917.

of the blood change arsphenamin into an insoluble base with the production of emboli of precipitates, which later may be dissolved in part or entirely by the leukocytes and organic bases of the plasma; also in view of the theory of Berman,⁷ that an increase of the globulin fraction of the serum proteins in certain persons may result in their precipitation in vivo. In studies on these subjects previously referred to⁸ the conclusion was reached that the theory of Danysz may be accepted as explaining in part the reactions caused by the intravenous injection of acid solutions of arsphenamin, to a lesser extent of concentrated monosodium solutions, but not at all of solutions of disodium arsphenamin, because of the negative character of all tests with the latter solutions.

As shown in the present studies, thrombi were found in practically all organs after the administration of solutions of acid and disodium arsphenamin; these thrombi were readily recognized as conglutinated masses of erythrocytes which later became hyalinized, due in part to hemolysis and autolysis. The characteristic and peculiar whorl-like construction and color of these masses caused by the administration of acid solutions indicates that they may have been composed of precipitates in addition to hemolyzing erythrocytes; the same is probably true of the thrombi found after the administration of single large doses of solutions of disodium arsphenamin and to a lesser extent of those thrombi caused by the intravenous injection of multiple small doses of disodium arsphenamin. The relation of hemolysis to these intravascular phenomena is especially significant as arsphenamin itself is hemolytic, especially acid and disodium solutions in water.⁹

Next to the vascular phenomena, varying degrees of degeneration and necrosis in parenchymatous organs were frequently conspicuous, especially among those animals surviving large amounts of arsphenamin for a sufficient time for necrotic changes to occur. Areas of necrosis of muscle cells were found in the heart; islands of necrotic liver and spleen were frequently conspicuous and pronounced nephrosis commonly occurred. While a portion of these degenerative and necrotic changes may reasonably be ascribed to the effects of thrombosis and embolism, one cannot escape the conclusion that solutions of arspen-

7. Berman, W.: The Nitritoid Crisis after Arsphenamin Injections, *Arch. Int. Med.* **22**:217, 1918.

8. Schamberg, J. F.; Kolmer, J. A.; Raiziss, G. W., and Weiss, C.: Laboratory and Clinical Studies Bearing on the Causes of the Reactions Following Intravenous Injections of Arsphenamin and Neo-Arsphenamin, *Arch. Dermat. & Syph.* **38**:235-256, 1920.

9. Kolmer, J. A., and Yagle, E. M.: Hemolytic Activity of Solutions of Arsphenamin and Neo-Arsphenamin, *J. A. M. A.* **74**:643 (March 6) 1920.

amin are capable of direct tissue injury of varying severity, according to the size of the dose, nature of the solution and to some extent of the species of animal.

The suprarenal changes of congestion and thrombosis, cellular degenerations and reduction in lipoid and chromaffin contents are particularly significant and indicate the important relation of suprarenal injury to arspenamin therapy and the possibility of a relation between changes in this organ and certain symptoms following at times the administration of this drug to human subjects. By reason of the practical importance of this subject, we are continuing this study by means of combined histologic and biochemical investigations.

It is to be emphasized that the maximum changes were produced by large doses of acid or nonneutralized solutions of arspenamin and by doses of solutions of disodium arspenamin from eight to twelve times greater than the largest single dose commonly administered to human beings at one time; also that the administration of multiple small doses analogous to those given in the treatment of syphilis, produced at the most such slight tissue changes as would not in all probability influence the function of the various organs. The latter naturally possess a greater practical significance and interest. As previously stated, these histopathologic changes have been produced in these experiments by arspenamin from three different laboratories; no differences were discernible in the effects according to the particular brand of arspenamin employed. The researches of Ullmann¹⁰ indicate that the prewar salvarsan of German manufacture produced lesions of similar kind and degree as those found by us and described in this report.

CONCLUSIONS

1. The intravenous injection of lethal doses of acid non-neutralized solutions of arspenamin in experimental animals produces widespread and severe vascular injury characterized by congestion, thrombus formation and hemorrhage; later cellular degeneration and necrosis take place.
2. The intravenous injection of single large doses of solutions of disodium arspenamin (ten times larger than the maximum amount administered to human beings at one time) produce severe vascular and tissue alterations, particularly in the liver, kidney, suprarenals and spleen.
3. The intravenous injections of multiple therapeutic doses of solutions of disodium arspenamin corresponding to doses of 0.6 gm. per

10. Ullmann, K.: Experimentelles zur arsenwirkung auf die organe, Wien. klin. Wchnschr. **27**:838-843, 1914.

60 kilos of body weight, produce inconspicuous tissue alterations that do not appear to be sufficiently pronounced to interfere with the functions of the organs.

4. Detailed descriptions of the histologic changes found in the brain and meninges, heart, lungs, liver, kidneys, suprarenals and spleen are given.

5. These changes were induced by arsphenamin prepared by several different laboratories.

6. These histopathologic changes probably bear an important relation to arsphenamin reactions and to methods of arsphenamin therapy.