

## THE RELATIONSHIP OF CHRONIC PROTEIN INTOXICATION IN ANIMALS TO ANAPHYLAXIS.<sup>1</sup>

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The experiments which furnish the subject of this paper represent a continuation of some observations made upon the effect of repeated injections of foreign protein in animals sensitized to these specific proteins.<sup>2</sup> Under these conditions areas of degeneration with extensive inflammatory reaction are produced in the myocardium, the liver, and the kidneys. In different species of animals the effects vary somewhat, since the kidney is involved in the dog, cat, rabbit, and guinea pig, the liver in the rabbit and cat,<sup>3</sup> and the myocardium<sup>4</sup> in the rabbit and guinea pig. A detailed description of these changes has already been published, and it is, therefore, necessary only to state that they consist in focal areas of degeneration, infiltrated with small round cells, which in the heart are scattered throughout the myocardium of both ventricles, in the liver are usually situated about the portal spaces, and in the kidney are seen most often through the midzone, whence they extend into the cortex. In their most advanced stages these alterations produce the picture of an extensive myocarditis, a periportal cirrhosis of the liver, or a wide-spread subacute nephritis with involvement, at times, of the glomeruli.

At first it was thought that sensitization to some foreign protein, such as horse serum or egg-white, was necessary before subsequent injections of the same protein could bring about the development of such changes, but further study showed that similar, though

<sup>1</sup> Read before the Association of American Physicians, Washington, May 10, 1915.

<sup>2</sup> Longcope, W. T., *Jour. Exper. Med.*, 1913, xviii, 678.

<sup>3</sup> Longcope, *Tr. Assn. Am. Phys.*, 1913, xxviii, 497.

<sup>4</sup> Longcope, *Arch. Int. Med.*, 1915, xv, 1079.

less marked, alterations might occur in animals receiving but a single large dose of horse serum or egg-white. The question, therefore, arose as to whether the effects produced were the result of introducing repeatedly into the bodies of these animals small quantities of a substance which was in itself primarily toxic for the cells of the kidneys, liver, and myocardium, or whether the horse serum and egg albumen became injurious only after the animals were sensitized to these substances. In the latter case the subacute inflammatory reactions might readily be dependent upon the repeated anaphylactic shocks to which the animals were subjected.

Though a number of observers interested principally in the fact that egg-white, when introduced parenterally into animals, is eliminated as such in the urine, have studied casually the kidneys of animals soon after an injection of egg-white, very few have described definite alterations in the kidneys. Adams<sup>5</sup> and Maschke<sup>6</sup> state that the kidneys of rabbits after single injections of egg-white are normal. Sollmann and Brown<sup>7</sup> observed under the same conditions occasional swelling and degeneration of the tubular epithelium. Chiray<sup>8</sup> could not discover any changes in the kidneys of rabbits after a single intravenous, intraperitoneal, or subcutaneous injection of egg albumen, but noted after repeated injections, which were limited to a few animals, degeneration of the tubular epithelium with cellular reaction and scar formation that led to chronic nephritis.

The following experiments were undertaken with the view of determining, if possible, whether the toxic action of horse serum and egg-white was a primary property of these proteins or developed only when the animals were sensitive to such foreign proteins. The latter view would express the idea which has long been held by Vaughan.<sup>9</sup>

It was thought that if the protein itself were toxic, it might be possible to demonstrate this toxicity by injecting repeatedly at weekly intervals proteins always of a different type, so that the animal might receive large quantities of foreign protein but never the same protein twice, thereby avoiding anaphylactic shock.

The following experiment was, therefore, performed:

<sup>5</sup> Adams, B., Inaugural Dissertation, Leipzig, 1880.

<sup>6</sup> Hirsch, C., and Maschke, W., *Berl. klin. Wchnschr.*, 1912, xlix, 145.

<sup>7</sup> Sollmann, T., and Brown, E. D., *Jour. Exper. Med.*, 1902, vi, 207.

<sup>8</sup> Chiray, M. M., Thèse de Paris, 1906.

<sup>9</sup> Vaughan, V. C., Vaughan, V. C., Jr., and Vaughan, J. W., *Protein Split Products in Relation to Immunity and Disease*, Philadelphia and New York, 1913.

Four lots of rabbits were selected from a single stock. Lot A, consisting of 11 rabbits, was used as control. Lot B, consisting of 11 rabbits, was sensitized to horse serum or egg-white by intraperitoneal inoculation of 2 to 4 cc. of these proteins on 3 consecutive days. After an interval of several weeks they were reinoculated intravenously at weekly intervals with horse serum in amounts varying from 0.5 to 6 cc., or with egg-white in amounts varying from 0.4 to 0.8 cc. Lot C, consisting of 9 rabbits, was injected intravenously at weekly intervals with horse serum, egg-white, beef serum, sheep serum, dog serum, cat serum, human serum, pig serum, edestin, and casein. The edestin and casein were made up in 2 per cent solution in sodium carbonate. The proteins were given in doses of 1 cc. per kilo of body weight, the actual dose varying from 1 to 2.7 cc. It was so arranged that the total amount of serum, etc., which each rabbit in this group received was as great or greater than that given to the animals in Group B.

Finally, in Lot D, a series of 17 rabbits was given each a single dose of horse serum or egg-white, either intravenously or intraperitoneally, and killed at intervals varying from 3 to 29 weeks. 5 of these received 2 cc. of egg-white intraperitoneally. 4 received 2 cc. of horse serum intravenously, and 1 rabbit 1 cc. of horse serum intravenously; 2 rabbits received 2 cc. intraperitoneally, and 1 rabbit received 1 cc. intraperitoneally; 1 received 3 cc. and 1 received 5 cc. of sheep serum intravenously; 1 rabbit received 6 cc. of dog serum intravenously and 1 received 6 of cat serum intravenously.

In order to eliminate any possible effect which fresh serum might have, the sera for all these experiments were heated for one-half hour at 56° C.

Table I gives the results of these experiments.

TABLE I.

Animals and tissues examined.	Lot A. Normal control rabbits. Uninoculated.	Lot B. Rabbits sensitized to egg-white or horse serum and after 3 weeks reinjected with egg-white or horse serum.	Lot C. Rabbits receiving repeated inoculations of different proteins (horse serum, egg-white, beef, sheep, dog, cat, human, and pig serum, edestin, and casein).	Lot D. Rabbits receiving a single dose of foreign protein.
No. of rabbits . . . . .	11	9	9	17
No. of intoxicating doses . . . . .	0	2-10	1-10	1
Involvement of myocardium . . . . .	0	9	3	7
Involvement of liver . . . . .	0	9	1	4
Involvement of kidneys . . . . .	1	9	3	12
Total No. of animals . . . . .	11	9	9	17
Total No. positive results . . . . .	1	9	3	12

The uninoculated control rabbits (Lot A) all showed normal organs except one. In this one rabbit a few areas of round cell infiltration were seen in the pelvis of the kidneys.

Of the eleven sensitized rabbits in Lot B, two died before intox-

icating doses of protein were administered. They showed normal organs. The nine sensitized rabbits receiving subsequent injections of serum all showed extensive and advanced changes in the heart muscle, liver, and kidneys.

In Lot C, only one-third of the animals developed changes in one or the other of these organs, and these were much less extensive than the lesions appearing in Lot B.

In Lot D, where the animals received but a single inoculation of egg-white or horse serum, the results corresponded quite well to those observed in Lot C.

It is, therefore, evident that though previous sensitization increases the incidence and severity of the changes, it is not necessary for their development.

During further experiments it was noted that when alterations occurred in the internal organs after a single inoculation they did not make their appearance for several days after inoculation. Experiments were, therefore, designed to determine more accurately the time of appearance of these lesions and to determine why this should be so long delayed. If the lesions were dependent upon the introduction of a toxic substance, one would expect, especially when the toxin was given in large amounts, that it would act much more promptly.

It is well known that the skin of an animal sensitized to a foreign protein such as horse serum will react specifically to the intracutaneous injection of that protein. In rabbits this specific sensitiveness of the skin usually develops within two to twenty days after the primary inoculation of horse serum, and, as Knox, Moss, and Brown<sup>10</sup> have shown, manifests itself after the intracutaneous injection of minute quantities of serum by the appearance in twelve to thirty-six hours of a large area of reddening, swelling, and edema, which persists at the site of inoculation for twelve to twenty-four hours. According to the view which generally prevails, the reaction is associated with the union of the antigen (horse serum) with antibodies formed against this specific protein.

This method was, therefore, utilized to determine whether or

<sup>10</sup> Knox, J. H. M., Moss, W. L., and Brown, G. L., *Jour. Exper. Med.*, 1910, xii, 562.

not the lesions in the internal organs following a single inoculation of horse serum were associated with the appearance of skin sensitiveness and, therefore, probably with the development of antibodies towards horse serum in the body of the animal.

It was at first hoped that repeated intracutaneous injections of horse serum in amounts varying from 0.02 to 0.1 cc. might be made, in order that the exact time at which sensitization developed could be fixed, but in normal rabbits used as controls the repeated intracutaneous injections, at four to six day intervals, of horse serum even in such small amounts led eventually to a positive reaction, which was interpreted as a true Arthus phenomenon. In such animals, however, general sensitiveness did not develop, since the injection of large quantities of horse serum intravenously in animals giving a positive skin test did not produce symptoms of anaphylaxis. In view of this fact it was possible to use the intracutaneous test only once.

For the final experiment 15 white rabbits were injected intravenously with from 5.5 to 9.0 cc. of horse serum in divided doses during 3 consecutive days.

TABLE II.

*Association between the Development of Lesions in the Heart, Liver, and Kidneys and the Appearance of Skin Sensitiveness in 15 Rabbits Receiving Single Injections of Horse Serum.*

Rabbit No.	Amount of serum.	Lived after injection.	Skin reaction.	Heart.	Liver.	Kidney.
	<i>cc.</i>	<i>days</i>				
15	5.5	7	Not made	0	0	0
9	8.5	8	" "	0	0	0
17	5.5	8	+	0	0	0
10	8.5	10	+	0	+	++
7	8.5	12	-	0	0	0
8	8.5	12	+	0	0	+
11	5.5	13	Not made	0	0	0
5	8.5	14	-	0	0	0
6	8.5	14	+	0	+?	0
13	5.5	17	+	0	0	+
16	5.5	21	-	0	0	0
18	5.5	22	- 20 hrs.	+	+	++
12	5.5	27	++	++	+	0
1	8.0	30	++	+	+	++
2	9.0	30	+	+	++	+
19 (control)	0			0	0	0
20 "	0			0	0	0
4 "	0		+ pure Arthus	0	0	0

At varying intervals after the first dose an intracutaneous injection of from 0.02 to 0.05 cc. of horse serum was made on one side of the shaved abdomen, while an equal amount of sheep or beef serum was injected in the skin of the opposite side as a control. The animals, except in one instance, were observed for from 36 to 48 hours and after the result of the skin test had been noted, killed, carefully autopsied, and their organs examined histologically.

The result of this experiment is given in Table II.

It will be seen that from the seventh to the twenty-first day after inoculation there were occasionally observed scattered and slight changes in the liver or kidney. When such alterations were found during this period they always occurred in rabbits that gave a positive skin test. All animals that gave a positive skin test, however, did not show changes in the internal organs.

After the twenty-second day the incidence and extent of the changes in the internal organs increased greatly and the skin reaction, except in one animal, was very intense. Uninoculated controls and one animal subjected to intracutaneous inoculation alone showed no changes in the internal organs.

Table II shows very well that the lesions in the internal organs appear either at the time the skin sensitiveness has made its appearance or directly afterwards, and therefore, after the animal has, hypothetically, produced antibodies to the foreign protein. One may liken the condition to serum disease in man during which time profound and very obvious disturbances may take place. In the rabbit the signs and symptoms so characteristic of the disease in man are lacking, though in one or two of the rabbits in this series moderate subcutaneous edema and generalized enlargement of the lymph nodes was observed at autopsy. Von Pirquet and Schick<sup>11</sup> have noted under the same conditions in rabbits alterations in the total and differential leucocyte counts that are analogous to those that are observed in children during serum sickness. The effect of anaphylactic shock is, however, so unlike in different species of animals that it is not permissible to draw general conclusions from the results obtained in any one species.

<sup>11</sup> von Pirquet, C. F., and Schick, B., *Die Serumkrankheit*, Leipzig and Vienna, 1905.

## CONCLUSIONS.

Such foreign proteins as horse serum and egg-white in the amounts employed in these experiments do not produce evidences of intoxication immediately after injection into rabbits. Single large injections do, however, produce changes in the parenchymatous organs after a period of ten to twenty-one days. These develop at the time or immediately after the animal has formed antibodies for the foreign proteins.

The mechanism of the development of the lesions in the myocardium, liver, and kidneys of rabbits is thus the same, whether a single inoculation is given or whether repeated inoculations are made in sensitized animals. By the latter method, however, much more marked and extensive changes may be produced.