

the hemagglutination and complement fixation titers of mumps infected allantoic and amniotic fluids and concluded that the hemagglutination titer was a function of virus concentration. Ginsberg, Goebel and Horsfall¹¹ showed that the rate of increase in concentration of virus, as determined by infectivity titrations, paralleled the rise of hemagglutination titers. The latter workers also found that an E.I. 50 titer of the order of $10^{-4.30}$ or higher was necessary before hemagglutination with mumps virus was demonstrable.

In order to rule out the possibility of a virus concentration titer too low to be detected by hemagglutination, the allantoic fluids of

the glycolized air samples were reinoculated into chick embryos. Following this second passage, no rise in titer was observed, and it was therefore concluded that no mumps virus was present.

Summary. 1. Under the conditions of these experiments, triethylene glycol vapor in saturated concentrations was rapidly lethal for air-borne mumps virus and Newcastle disease virus.

2. Triethylene glycol vapor was also lethal for air-borne mumps virus in concentrations below saturation.

We should like to express our indebtedness to Dr. Robert Ward for his helpful advice throughout this study.

¹¹ Ginsberg, H. S., Goebel, W. F., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, **87**, 385.

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17299. Effect of Ammonium Chloride on Serum Sodium/Chloride Ratio in Foreign Serum Arteritis in Rabbits.

CHESTER R. McLEAN AND ROBERT H. MORE.

From Department of Pathology, Pathological Institute, McGill University, Montreal, Canada.

An arteritis has been induced in rats by the administration of large amounts of desoxycorticosterone acetate (D.C.A.) by Selye,¹ and in rats so treated the development of these lesions was inhibited when NH_4Cl was added to their diet.¹ D.C.A. overdosage in rats also causes a rise in the serum sodium and the serum Na/Cl ratio^{1,2} and a reduction of this ratio toward the norm following NH_4Cl therapy has been held to be significant in inhibiting the development of D.C.A. induced arteritis and other lesions in the rat.¹ It was thought that if this elevation of the serum Na/Cl ratio was an essential step in the pathogenesis of the D.C.A. arteritis, then if there is a common pathogenesis in the development of the D.C.A. arteritis and the well known foreign protein arteritis,³ there should

also be an elevation of the serum sodium and Na/Cl ratio in animals given foreign protein which develop arteritis. It also seemed a logical possibility that ammonium chloride might inhibit the development of foreign protein arteritis if there is a common pathogenesis in the development of these two types of arteritis. The following experiments were designed to determine whether these logical possibilities concerning the pathogenesis of these two types of arteritis are correct.

Materials and methods. Group 1. Eighteen albino rabbits of 2-3 kg weight were given a diet of Purina Rabbit Chow and water to drink *ad libitum*. The rabbits were given an intravenous injection of sterile whole horse serum in a dose of 10 cc/kg of body weight. The serum injection was repeated after 17 days. Seven days later all animals were killed by air embolism and sections were prepared for histological study from all tissues as in our previous studies.³

Group 2. Seventeen albino rabbits of 2-3 kg weight derived from the same sources and

¹ Selye, H., Hall, O., and Rowley, E. M., *Lancet*, 1945, **248**, 301.

² Friedman, S. M., Polley, J. R., and Friedman, C. L., *J. Exp. Med.*, 1948, **87**, 329.

³ More, R. H., and McLean, C. R., *Am. J. Path.*, 1949, **25**, 413.

TABLE I.
 Serum Sodium/Chloride Ratios Estimated in Milliequivalents.

		A	B	C	D
Group I Received serum only	Mean	1.50 $\pm$.03	1.49 $\pm$.02	1.41 $\pm$.03	1.47 $\pm$.01
	S. Dev.	.10	.06	.08	.04
Group II Received serum and NH ₄ Cl	Mean	1.51 $\pm$.03	1.39 $\pm$.03	1.32 $\pm$.01	1.31 $\pm$.04
	S. Dev.	.08	.09	.05	.14

A. Before any treatment was given.

B. Immediately before the second serum injection.

C. Two days after the second serum injection.

D. Immediately before termination of the experiment.

living in the animal quarters at the same time were treated in exactly similar manner except that they were given a 0.9% ammonium chloride solution instead of water to drink. The salt solution was supplied in quantities of 300 cc daily; the residue, if any, from each 24 hour period was measured. This salt solution was well tolerated, although the majority of animals gave evidence of thirst and drank most of their daily quota within a few hours of being fed. Ammonium chloride was given for one week before the first injection of horse serum and continued until the end of the experiment.

Serum sodium and chloride levels in 10 animals from each group were determined on 4 occasions: A—before any treatment was given; B—immediately before the second serum injection; C—two days after the second serum injection; D—immediately before termination of the experiment. Sodium determinations were done according to the method of Barber and Koltoff⁴ as modified by Butler and Tuthill,⁵ and the chloride determinations were done by the method of Wilson and Ball.⁶

Results. The means, standard deviation, and standard error of the means of the serum Na/Cl ratio expressed as milliequivalents, are shown in Table I. The serum sodium was not appreciably affected either by serum treatment or the addition of ammonium chloride to the diet or both. There was no elevation of the Na/Cl ratios of the Group I animals receiving serum alone as occurs in

rats given large amounts of D.C.A. The individual animals of this group which developed lesions and on which serum sodium and chloride determinations were made showed no elevation of the serum Na/Cl ratio. In the animals of Group 2 receiving both serum injections and ammonium chloride in their drinking water, there was a progressive elevation of the mean serum chloride, and this was reflected in a depression of the Na/Cl ratio. This was most marked following the second injection of serum, when it reached levels that were significantly lower than normal values for this group of animals before treatment or values in the Group I receiving serum alone determined at corresponding times. Five of the sodium and chloride determinations made on animals from Group 2 receiving both foreign protein and ammonium chloride were made on animals that developed arterial lesions. In two of these the Na/Cl ratio was depressed significantly below normal values.

Focal inflammatory lesions were found in the coronary and visceral arteries of both groups. The arterial changes were qualitatively similar in both groups of animals. These were comparable to the lesions produced by injections of horse serum that we have described in detail elsewhere,³ and illustrated in Fig. 1. However, there was some difference in the incidence of the arterial lesions between the animals of Group 1 which received horse serum only and those of Group 2 which received both horse serum and ammonium chloride as shown in Table II. Arterial lesions were seen in 10 animals of Group 1 and in 6 of the ammonium chloride treated series. The total number of organs exhibiting arterial lesions in the animals of Group 1 was 23,

⁴ Barber, H. H., and Kolthoff, I. M., *J. A. Chem. Soc.*, 1928, **50**, 1625.

⁵ Butler, A. M., and Tuthill, E., *J. Biol. Chem.*, 1931, **93**, 171.

⁶ Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.

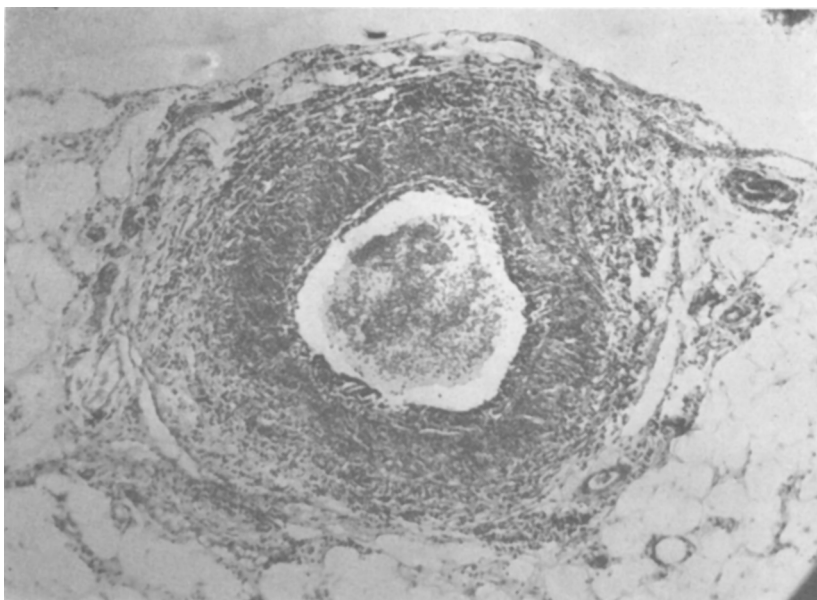


FIG. 1.

Typical arteritis following two large intravenous injections of horse serum, presenting fibrinoid necrosis of the media with cellular exudation into the intima, media and adventitia consisting, essentially, of large mononuclear cells.

Hematin phloxine and saffron $\times 90$.

whereas that in the ammonium chloride treated group was only 11.

Five of the animals of Group 1 and 6 of those of Group 2 showed focal inflammatory lesions of the heart valves. Morphologically, those of the NH_4Cl treated group were indistinguishable from those of Group 1.

Discussion. Selye¹ has postulated that one important factor in the pathogenesis of D.C.A. induced arteritis is the elevation of serum sodium with consequent elevation of the serum Na/Cl ratio. In order to establish the possible relationship between the pathogenesis of D.C.A. induced arteritis and foreign serum protein arteritis, it seemed to us important to establish whether this same relationship of elevated serum Na/Cl ratios and the development of arteritis held for the production of foreign serum protein arteritis. In Group 1 receiving serum only, our results show that arteritis occurred without elevation of the serum sodium or serum Na/Cl ratio. It is clear then that if the elevation of the serum Na/Cl ratio is an essential factor in the pathogenesis of D.C.A. induced arteritis, then the pathogenesis of foreign protein arteritis

must be different. Further evidence that an elevation of the Na/Cl ratio is not important in the development of the foreign protein arteritis is indicated by the fact that in the animals receiving both horse serum and ammonium chloride two animals, at least, that developed arteritis, showed a significant reduction in the sodium chloride ratio below the mean normal value and significantly below the control pre-treatment values in these two rabbits. However, in view of the observations of Darrow and Miller⁷ that the mineral content may vary independently in the serum and tissues, the possibility still exists that in the present experiment there may have been alterations in the sodium content of the tissues undetected by analysis of serum sodium.

The interpretation of the possible inhibitory effect of ammonium chloride on the development of the arteritis is not so clear. There was a decrease in the total number of affected animals and in the total number of organs with lesions in Group 2, but this decrease was not statistically significant when

⁷ Darrow, D. C., and Miller, H. C., *J. Clin. Invest.*, 1942, **21**, 601.

TABLE II.
Comparison of Incidence of Arteritis Between Group I and II.

Arteries	Incidence in organs		Incidence in animals	
	Group I Serum only 18 rabbits	Group II Serum and NH_4Cl 17 rabbits	Group I Serum only 18 rabbits	Group II Serum and NH_4Cl 17 rabbits
Coronary	10	5		
Aorta	6	2		
Mesenteric	2	2		
Gastric	1	1		
Pancreatic	4	1		
Total	23	11	10	6

compared with the incidence of lesions in the animals of Group 1 receiving horse serum alone. Whether the trend might become significant by a more effective use of ammonium chloride is not established from the results, but it seems possible for the following reason. The depression of the Na/Cl ratio did not reach significant levels until after the 24th day of the treatment and this fact may indicate that any effect of ammonium chloride on the animals as a whole was not reached until this time. It therefore seems possible that if ammonium chloride (by whatever mechanism) is responsible for the lowered incidence of lesions seen in Group 2, then more prolonged treatment with ammonium chloride before the animals receive foreign protein injections might be more effective in inhibiting the development of these lesions, and the trend toward inhibition of arteritis in the rabbits receiving both horse serum and ammonium chloride might reach significant levels. If this latter possibility were the case it might indicate a common pathogenesis for the development of the D.C.A. induced arteritis on the one hand and foreign protein induced arteritis on the other, but from what has already been said, the alteration of the Na/Cl ratio could not be an essential step

in such a common pathogenesis.

Summary. The possibility was considered that experimentally induced D.C.A. arteritis and foreign protein arteritis might have a common pathogenesis mediated by way of an elevation of the serum Na/Cl ratio. To test this hypothesis one group of animals was given horse serum alone, and another both horse serum and ammonium chloride. There was no elevation of the serum sodium or Na/Cl ratio in those animals receiving horse serum alone and some of the animals receiving both horse serum and ammonium chloride showed a significant depression of the Na/Cl ratio, even though they developed an arteritis.

These results clearly indicate that the development of foreign protein arteritis is not mediated through alterations of the serum sodium chloride ratio and therefore if such an alteration is essential to the development of D.C.A. induced arteritis as suggested by Selye,¹ then the pathogenesis of these two varieties of arteritis must be different. There was a decreased incidence of arteritis in the group of animals receiving horse serum and ammonium chloride, and it is suggested that this trend might become significant by a more effective use of ammonium chloride.

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