

mation show that there is a decrease in total available pepsin and in pepsin concentration, but only a decrease in total available gastric acidity and not in its concentration. Total available lysozyme was decreased

but not its concentration. The volume of gastric juice was decreased while its viscosity was increased. Total available mucin was increased as was its concentration.

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Effects of Cortisone, Hyaluronidase, Desoxycorticosterone, and Artisone on Experimental Serum Disease in Rabbits. (18191)

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In previous experiments it was shown that large doses of colchicine or nitrogen mustard depress or prevent the Arthus reaction in the skin and the allergic arteritis and valvulitis caused by horse serum in rabbits(1,2). As this was associated with depression of precipitin formation, and enlargement of the adrenals and shrinkage of the thymus (alarm reaction), it was postulated that the beneficial effect of the drugs in this disease was due to destruction of antibody forming cells through the mediation of the adrenal cortices(1). Recently it was demonstrated that 10 to 20 mg of adrenocorticotrophic hormone (ACTH) per day likewise depressed the Arthus reaction, allergic arteritis and endocarditis, the weight of the thymus, and precipitin formation(3). There is reason to believe that these effects are produced by the glucocorticoids, which also influence permeability of the ground substance(4), the site extensively involved in serum disease. We considered it desirable, therefore, to compare the effect in serum disease of cortisone, which causes both lymphoid involution and changes in permeability of the ground substance, with

hyaluronidase, desoxycorticosterone acetate (DCA), and 21-acetoxypregnenolone (Artisone), which also alter ground substance permeability but do not affect lymphoid tissue.

Materials and methods. All experiments were performed in young male albino rabbits weighing between 1760 and 2500 g. One series of 40 rabbits was given by intravenous injection 15 ml of normal horse serum without preservative per kg of body weight. Three groups of 10 animals each received 4 mg cortisone acetate,* 2.5 mg DCA,† and 5 mg Artisone acetate‡ twice daily by intramuscular injection; the remaining 10 animals served as serum controls. Another series of 20 rabbits received 10 ml horse serum per kg. Ten of these received 6000 turbidity reducing units (T.R.U.) of hyaluronidase§ per kg of body weight (twice daily by intravenous injection); the other 10 served as serum controls. Half the rabbits of each group were killed 15 days after the first injection of serum. The other half received a second injection of serum 19 days after the first and were sacrificed 5 days later. Three additional rabbits received hyaluronidase only (6000 T.R.U. per kg twice daily by intravenous

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2. Dammin, G. J., and Bukantz, S. C., *J. Am. Med. Assn.*, 1949, v139, 358.

3. Berthrong, M., Rich, A. R., and Griffith, P. C., *Bull. Johns Hopkins Hosp.*, 1950, v86, 131.

4. Seifter, J., Baeder, D. H., and Bogany, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 277.

* Saline suspension of Cortone Acetate, Merck.

† Cortate, oil solution. We are indebted to the Schering Corp. for furnishing some of this material.

‡ Micronized aqueous suspension of 21-acetoxypregnenolone, Wyeth.

§ Hydase, Wyeth.

TABLE I. Average Size and Incidence of Arthus Reaction in Skin 24 Hr After Intradermal Inj. of 0.1 ml Horse Serum on 14th Day of Experiment.

	Positive reactions, %	Size of positive reactions, cm
Control Serum	0	0
" + DCA	90	2.94×3.39
" + Artisone	87.5	2.42×2.67
" + cortisone	100	2.93×3.43
Control Serum	78	2.33×2.67
Hyaluronidase	0	0
" + hyaluronidase	0	0
" + hyaluronidase	100	4.7×5.8
" + hyaluronidase	70	2.71×3.43

injection). and were killed 24 days after the initial injection. Eight rabbits served as normal controls.

Results. One each of the hyaluronidase and Artisone treated, 2 of the DCA treated, and 5 of the cortisone treated rabbits died during the course of the experiment. The Artisone and DCA treated animals died with pulmonary edema. Three of the cortisone treated rabbits showed suppurative pneumonia, pleurisy, and pericarditis; 4 revealed multiple necrosis in the liver. The Arthus reaction in the skin elicited by intradermal injection of 0.1 ml horse serum was depressed by cortisone (Table I). A similar depression occurred with DCA and hyaluronidase but not with Artisone. Proteinuria was not present in any of 22 serum or steroid treated rabbits of the first series examined on the eleventh day after the first injection of serum. A second group of 13 rabbits of this series were killed on the fifteenth day at which time 2 rabbits of 4 receiving serum only and 1 of 3 DCA treated ones had 60 to 120 mg of protein per 100 ml of urine as measured by the Exton test(5). Sixteen rabbits of the previously mentioned 22 were killed 5 days after the second injection of serum and the urine was retested. At this time only the cortisone treated animal had proteinuria (70 mg per 100 ml). The extra-capillary glomerular nephritis was mild in the cortisone treated animal and severe in the 3 other rabbits with proteinuria.

The changes in weight of the various organs following injection of 15 ml horse serum per kg of body weight are shown in Tables II and III. The tremendous increase in weight of the spleen 15 days after the first injection of serum was due largely to increased activity of the germinal centers; 5 days after the second injection proliferating plasma cells were an important factor in the enlargement. Kidneys, lungs, and heart were also markedly enlarged, apparently due to the mesenchymal changes observed in these organs.

On microscopic examination the incidence of arteritis, carditis, and glomerular nephritis was observed to be greater with 15 ml horse serum per kg than with 10 ml. It is also of interest that the albino rabbits used in this experiment had more severe serum disease than was observed in the chinchilla variety used in previous studies. Furthermore, albino rabbits receiving the 15 ml dose of horse serum showed necrotizing arteritis and extra-capillary glomerular nephritis even after the first injection (3 of 14 rabbits on the fifteenth day), whereas in 41 chinchilla rabbits such lesions were not seen after the first injection of 15 to 20 ml horse serum per kg but only after the second injection.

The arteritis was most severe in the heart, lungs, and spleen, next in the pancreas and liver, and least in the thymus and thyroid. The myocardium and heart valves were more severely involved than the endocardium and pericardium. In the lungs the small vessels were much more affected than the large ones. In the kidneys the glomeruli were especially affected. Fifteen days after the first injection the spleen was crowded with large active germinal centers, while 5 days following the second injection these were markedly depressed and abundant plasma cells were found in the spleen and liver.

The administration of hyaluronidase to serum treated rabbits caused a greater frequency and severity of arteritis and carditis, but less glomerular nephritis (Table IV). However, following the second injection of serum no significant difference was noted between the hyaluronidase treated animals and the serum controls. Precipitin ring tests were

5. Exton, W. G., *J. Lab. and Clin. Med.*, 1925, v10, 722.

TABLE III. Percentage Changes in Weight of Organs (g/kg) as Compared with Organs of Normal Rabbit.

	No. of rabbits	Heart	Lungs	Kidneys	Liver	Spleen	Thymus	Adrenals
15 days after first inj. of serum								
Serum	4	+ 16	+22	+23	+ 1	+203	— 3	— 8
" + DCA	4	+ 39	+16	+43	— 7	+203	—11	—11
" + Artisone	4	+ 26	+ 9	+39	—10	+268	+21	—14
" + Cortisone	8	+105	+30	+66	+ 74	+ 26	—77	—30
5 days after second inj. of serum								
Serum	5	+ 16	+23	+29	+ 1	+332	—17	— 2
" + DCA	4	+ 45	+56	+58	+ 1	+208	—69	+14
" + Artisone	4	+ 55	+ 4	+ 8	— 1	+200	—60	+12
" + Cortisone	1	+ 46	+13	+48	+101	+ 14	—68	— 3

TABLE IV. Frequency (a)* and Intensity (b) of the Various Lesions Caused by Serum.

	No. of rabbits	Heart						Lungs				Kidneys			

* See footnote, Table II.

positive with the sera of all the hyaluronidase treated rabbits (0.2 ml of a 1:3 dilution of serum overlaid by 0.2 ml of a solution containing 50 to 0.5 T.R.U. of hyaluronidase) and negative with the sera of the serum controls.

The treatment with cortisone, on the other hand, had a remarkable effect on almost all lesions (Tables II and III). It prevented all vascular changes with the exception of those in lungs and spleen in the rabbit killed 5 days following the second injection of serum. It caused a marked decrease in adrenal weight, due to atrophy of the zona fasciculata, and in the weight of thymus and spleen, due to lymphocytolysis. There was a remarkable increase in the weights of liver, kidneys, and heart, apparently due to the deposition of glycogen. The glycogen content of the liver from the animal sacrificed 5 days after the

second injection of serum was more than twice that of the controls (Table V). However, glomerular nephritis was not prevented; 8 of the 9 cortisone treated rabbits had up to 4 plus nephritis, intra-capillary as well as extra-capillary.

DCA and Artisone had no remarkable effect on the histological manifestations of serum disease except that the livers and adrenals of the animals receiving DCA revealed marked extra-medullary myelopoiesis. However, following the second injection of serum, the adrenals were larger and the thymus and spleen smaller than those of the serum controls, suggesting that the drugs in the dosages used had a mild alarm reaction effect. There was also a marked drop in the glycogen content of the liver in the DCA treated rabbits (Table V). No attempt was made to determine whether the doses of DCA as large as those

TABLE V. Glycogen Content of Liver 5 Days After Second Inj. of Serum. (Anthrone method).*

	No. of rabbits	Body wt, kg	Liver wt, g	Glycogen, mg/100 mg
Control	2	—	94.3	1.94
Serum	5	2.58	100.3	1.73
" + DCA	4	2.44	104.2	0.56
" + Artisone	5	2.58	99.6	1.85
" + Cortisone	1	2.53	195.7	3.75

* Seifter, S., Dayton, S., Novic, B., and Muntwyler, E., *Arch. Biochem.*, 1950, v25, 191.

used by Selye to produce adaptation disease and whether larger doses of Artisone effect the severity and incidence of serum disease.

Discussion. The depression of Arthus reaction in the skin by DCA and hyaluronidase can be accounted for by the spreading action of hyaluronidase and a similar enhancement of permeability of the ground substance by DCA(4), either of which would cause more rapid dispersion of the injected antigen. Cortisone, on the other hand, like nitrogen mustard(2) and ACTH(3), acted probably by depressing antibody formation rather than by decreasing spread through the skin, for Artisone in equi-antipermeability dose had no effect on the Arthus reaction. The absence of proteinuria on the eleventh day as compared with the positive findings during the first week of serum disease described elsewhere(6) are in agreement with our conclusion that the proteinuria during the week following the first injection of serum is due to direct damage of the glomeruli by the serum rather than to allergic glomerular nephritis. The abundance of plasma cells in the spleen and liver seen soon after the second injection of serum is consistent with the concept that plasma cells are sources of antibodies. The presence of large active germinal centers in the spleen 15 days after the first injection of serum and the marked depression of these soon after the second injection supports the observation that the activity of the germinal centers follows the formation of plasma cells and antibodies(7).

It is conceivable that the enhancement of arteritis and carditis and lessening of glomerular nephritis by hyaluronidase was due to increased permeability of the ground substance by the enzyme thereby permitting more antigen and antibody to penetrate the walls of the vessels and thus diverting some of the load from the glomeruli. The results of the precipitin ring test suggest that the failure of hyaluronidase to enhance the lesions after the second injection of serum may have been due to neutralization of this enzyme by anti-hyaluronidase. This interpretation is supported by the observation that the huge doses of hyaluronidase *per se* caused myocarditis, vascular changes in the lungs, and glomerular nephritis (Table IV).

The mechanism by which cortisone suppressed the arteritis and carditis is not completely understood. It is apparent that the demonstrated depression of antibody formation through destruction of antibody forming cells(1-3) was a major factor. Other mechanisms probably of less importance also were involved since cortisone did not prevent glomerular nephritis. This latter observation is in accord with the clinical experience that cortisone has no beneficial effect in acute glomerular nephritis in humans. It is of considerable interest that one rabbit sacrificed 15 days following the first injection of serum but not included in Tables II and III because of a broken back, showed a unilateral 6+ extracapillary glomerular nephritis, the other kidney being essentially normal. In other respects this rabbit resembled the cortisone treated ones. The small adrenals (0.141 g per kg) and thymus (0.23 g per kg) and the absence of arteritis and carditis indicate that this animal had a severe alarm reaction. The decreased arteritis and carditis after cortisone and the opposite effect after hyaluronidase suggest that generalized depression of the permeability of the ground substance also were involved in the action of cortisone. The antipermeability effect of cortisone on ground substance has been observed(4,8). The failure of Artisone to af-

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7. Ehrich, W. E., Drabkin, D. L., and Forman, C., *J. Exp. Med.*, 1949, v90, 157.

fect the serum disease lesions does not support this assumption since the doses used depress ground substance permeability as effectively as cortisone(9), but larger doses would have to be administered before the permeability factor could be evaluated. The permeability of different ground substance structures may be affected to different degrees by various steroids.

These studies seem to show that serum disease is an excellent experimental tool for evaluating the total actions of cortisone-like drugs in alleviating diseases of the ground substance. Permeability studies of the joints previously described are useful as preliminary screening, for it has been found that all effective anti-rheumatic drugs suppress permeability of the synovial membrane.

Conclusions. Cortisone in doses of 4 mg twice daily depressed the Arthus reaction in the skin and completely prevented arteritis and carditis in 8 of 9 rabbits treated with

horse serum. Glomerular nephritis, on the other hand, was not prevented. The zona fasciculata of the adrenals was atrophied. The mortality rate was highest among the animals receiving cortisone. Hyaluronidase in doses of 6000 T.R.U. per kg depressed the Arthus reaction in the skin and diminished glomerular nephritis, but enhanced arteritis and carditis. These observations may be explained by the ability of hyaluronidase to weaken ground substance barriers, thereby diverting antigen and antibody from the kidneys to the general vascular bed. DCA and Artisone in the doses used had no remarkable effect on the histological manifestations of serum disease, but DCA caused a depression of the Arthus reaction in the skin possibly through its hyaluronidase-like effect upon the ground substance.

It appears that serum disease is an excellent experimental tool for comparing the total efficacy of cortisone-like drugs after preliminary screening for their anti-permeability action on ground substance.

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Histological Manifestations of Feeding Polyoxethylene Monostearates To Weanling Hamsters.*† (18192)

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It was reported recently(1) that the ingestion of 5 or 15% of polyoxethylene monostearate preparations (Sta-soft and Myrj) by weanling hamsters caused an increased mortality rate, a decrease in food efficiency and rate of gain as compared to similar animals fed 5 or 15% lard, or 7½% lard plus 7½% cellulflour in addition to the basal ration.

The experimental animals suffered a high incidence of diarrhea and other gross pathological manifestations. Similar results were obtained when other surface active agents were fed to hamsters and rats(2,3). In the present report histological changes observed in the organs of the hamsters from the investigations described previously(1) are presented.

Experimental. One animal fed either 5

* We are indebted to Mrs. Marjorie Ismond for assistance in preparation of the slides.

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2. Sherman, H., Harris, R. S., and Jetter, W. W., *Fed. Proc.*, 1950, v9, 370.

3. Harris, R. S., Sherman, H., and Jetter, W. W., *Ibid.*, 1950, v9, 361.