lent to 2 ml of the original plasma per 100 g body weight induced a 30% increase in the recipients' thrombocyte counts. Smaller doses, *i.e.*, 0.5 or 1 ml per 100 g body weight, did not promote thrombocytosis. All other hematologic measurements in these animals remained stable. These findings provide additional support for the hypothesis that thrombopoiesis is subject to humoral regulatory control.

1. Gordon, A. S., *Physiol. Rev.*, 1959, v39, 1.
2. Linman, J. W., Bethell, F. H., *Factors Controlling Erythropoiesis*, Charles C Thomas, Springfield, Ill., 1960.
3. Jacobson, L. O., Gurney, C. W., Goldwasser, E., in *Advances in Internal Medicine*, Year Book Publishers, Chicago, Ill., 1960, v10, 297.
4. Linman, J. W., Bethell, F. H., Long, M. J., *Ann. Int. Med.*, 1959, v51, 1003.
5. Linman, J. W., *J. Lab. and Clin. Med.*, 1962, v59, 262.
6. Kelemen, E., Lehoczky, D., Perkedy, J., Cserháti, I., Rák, K., *Lancet*, 1960, v1, 1134.
7. Spector, B., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 146.
8. Odell, T. T., Jr., McDonald, T. P., Detwiler, T. C., *ibid.*, 1961, v108, 428.
9. Reichlin, M., Harrington, W. J., *Blood*, 1960, v16, 1298.
10. Gurney, C. W., Goldwasser, E., Pan, C., *J. Lab. and Clin. Med.*, 1957, v50, 534.
11. Odell, T. T., Jr., McDonald, T. P., Detwiler, T. C., Howsden, F.L., *Blood*, 1961, v18, 796.
12. Schulman, I., Pierce, M., Lukens, A., Currimbhoy, Z., *ibid.*, 1960, v16, 943.
13. Schulman, I., Currimbhoy, Z., Fort, E., Alcalde, V., *Am. J. Dis. Children*, 1960, v100, 747.
14. Steinberg, B., Dietz, A. A., Martin, R. A., *Acta Haemat.*, 1959, v21, 78.

Received April 12, 1962. P.S.E.B.M., 1962, v110.

Conditioning Effect of Certain Minerals upon Development of Nephrotoxic Serum Arthritis in Rabbits.* (27551)

CONSTANTIN V. TEODORU AND BRUNO W. VOLK
(With technical assistance of Alice Einhorn)
Isaac Albert Research Institute, Jewish Chronic Disease Hospital, Brooklyn, N. Y.

It has been shown that repeated injections of small amounts of Duck Anti-Rabbit Kidney Serum (DARKS) into the joints of rabbits cause a progressive, self-perpetuating arthritis which develops after incubation periods averaging 10 weeks(1-3). Normal Duck Serum (NDS) given under similar circumstances fails to produce arthritis. Since the slow progress of the monoarthritis produced by DARKS is impractical for experimental purposes an attempt was made to accelerate its development by conditioning factors such as high intake of NaCl which had previously been shown to enhance the effects of DARKS in nephritis(4) and in arthritis (2).

The present paper describes the effect of high intake of NaCl and KCl upon the clinical and pathological evolution of the nephrotoxic serum arthritis.

* Supported by Nat. Inst. Health grant.

Material and methods. DARKS and NDS were prepared following the method previously described(1,5). Bacteriological cultures were frequently performed on all serum samples. Contaminated sera were eliminated. Duck serum globulins were isolated and concentrated from both DARKS and NDS following the alcohol precipitation method as described by Mellors(5).

Adult albino rabbits of either sex weighing 3-4 kg were separated into 3 main groups. A) One group of 26 animals was preconditioned with 0.9% NaCl given instead of drinking water. B) Another group of 18 rabbits was preconditioned with 1.14% potassium chloride given the same way. C) A third group of 26 rabbits drinking tap water, served as controls. Each group was divided into 3 equal subgroups which were treated as follows: 1) One-third of each group was injected with DARKS in the right knee. 2) The second

TABLE I. Incidence of Arthritis Following Intra-Articular Injections of Nephrotoxic (DARKS) or Normal (NDS) Duck Serum in Preconditioned and Control Rabbits.

Intra-articular	Preconditioning	No. of animals	Incidence of arthritis, %	Avg latent period, days	Period of development, days
DARKS	None	18	100	68	115
	NaCl	15	100	13	27
	KCl	9	100	19	34
	Duck serum i.d.	4	100	6	8
NDS	None	16	0	—	—
	NaCl	9	89	21	34
	KCl	6	83	25	39
	Duck serum i.d.	3	100	8	11

third received NDS in the right knee. 3) The remainder was injected with DARKS in the right and NDS in the left knee. D) An additional group of 7 rabbits preimmunized intradermally, then injected intra-articularly (i.a.) was used as control for various types of accelerated arthritis. The technic of i.a. injections has been described(1).

All rabbits were bled frequently before, during and after treatment. Presence of circulating antibodies against duck serum proteins was grossly estimated by the precipitin reaction occurring between rabbit and duck serum on one hand and between rabbit serum and isolated DARKS globulins on the other hand. The capillary tubes used for the reaction were incubated for 2 hours at 37°C, then refrigerated overnight. The precipitin reactions, estimated as 0-5+, were performed for each group twice weekly.

The incidence of arthritis was expressed as per cent for each group. Individual severity was quantitatively estimated as 0-5+. The latent period designated the time elapsed between the first i.a. injection of serum and the first symptoms of arthritis. The time interval between the first i.a. injection and the phase of fully developed arthritis was also averaged per group and called period of development. The animals were sacrificed by overdosage of nembutal and both knee joints were removed for gross inspection and microscopic examination. Various other organs including striated muscles, heart, lung and kidneys were also studied histologically.

Results. (A) *Non-conditioned rabbits.* DARKS injected i.a. (0.5 ml) into non-conditioned rabbits twice weekly for 6 weeks, provoked monoarthritis after a latent period averaging 10 weeks. The symptoms reached their peak in about 6 weeks from the first clinical symptoms (Table I). NDS injected similarly failed to induce arthritis for observation periods up to 21 weeks (Table I).

Non-conditioned animals injected with DARKS into one joint and NDS into the contralateral joint developed clinical signs of arthritis in the DARKS treated joint only(1).

(B) *Pre-conditioned rabbits.* When DARKS was injected i.a. in animals preconditioned with 0.9% sodium chloride, arthritis developed within 2 instead of 10 weeks as compared to non-conditioned controls. The clinical symptoms reached their peak within 2 weeks from their emergence (Table I). The conditioning effects were comparable regardless of whether saline was given by mouth (*i.e.*, instead of drinking water) or intra-articularly (*i.e.*, 0.5 ml injected twice weekly contralaterally to DARKS).

When NDS was injected into the joint of animals preconditioned with NaCl, 8 out of 9 animals developed arthritis within an average of 3 weeks (Table I).

In animals preconditioned with potassium chloride both DARKS and NDS produced arthritis after latent periods averaging 3 and 4 weeks respectively (Table I). Animals preimmunized 6 to 9 months previously with 2 intradermal injections of either DARKS or NDS developed arthritis within a week following i.a. injection of duck serum, regardless of whether DARKS or NDS was employed.

The clinical and morphologic features of arthritis were not only accelerated but also accentuated by preconditioning, particularly in the groups drinking saline. Clinically

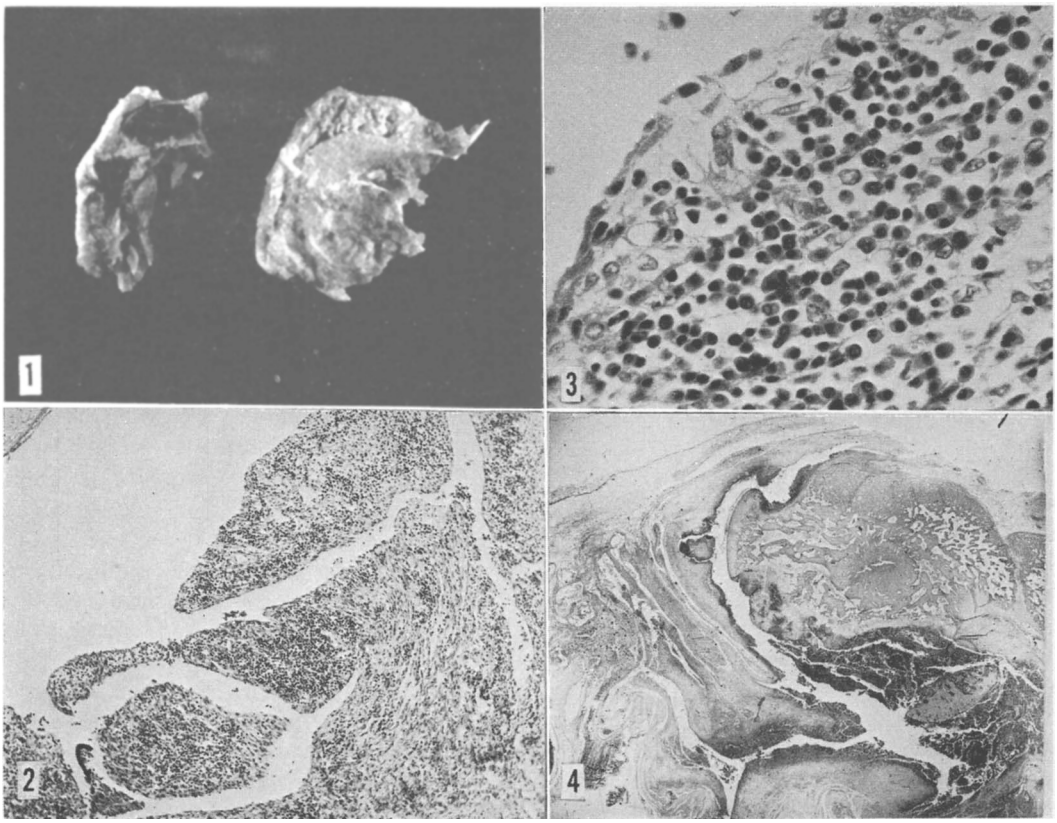

FIG. 1. Knee joints of preconditioned rabbit 23 wk after i.a. injections with DARKS (right) and saline (left). The DARKS-inj. joint is swollen and shows destruction of meniscus and articular cartilage. Synovial cavity contains necrotic debris. The saline-inj. joint, serving as control, is intact.

FIG. 2. Synovia of preconditioned rabbit 4½ wk after i.a. injections with DARKS showing villous hypertrophy and infiltration with lymphocytes, plasma cells and monocytes. H & E. × 44.

FIG. 3. High power field of Fig. 2, showing infiltrate of the synovium consisting of lymphocytes, plasma cells and monocytes. × 334.

FIG. 4. Knee joint of preconditioned rabbit 23 wk after i.a. injections with DARKS. There is fibrinoid necrosis of synovium and erosion of articular cartilage with exposure of underlying bone. Synovial cavity contains an exudate consisting of proteinaceous material, lymphocytes, plasma cells and small numbers of polynuclear leukocytes. H & E. × 3.33.

there was inflammation and swelling of the joint, marked functional impairment, tenderness of hamstring muscles and obvious pain at passive movements.

At autopsy there was destruction of the meniscus, necrosis of the articular cartilage and accumulation of necrotic debris in the synovial space (Fig. 1). The earliest histologic change in the synovium of the involved knee consisted of proliferation of the synovial cells. This was followed by proliferation of fibroblasts and capillaries as well as by infiltration of the synovia with lymphocytes, plasma cells and monocytes, resulting frequently in villous-like hypertrophy (Fig. 2, 3). Later the synovial membranes showed fibrinoid necrosis and the articular cavity contained an exudate which consisted of proteinaceous material, lymphocytes, plasma cells and small amounts of polynuclear leukocytes. Eventually membranes composed of necrotic material and round cells were seen to cover the articular cartilage which often became eroded, so that the subchondral bone was exposed (Fig. 4).

In the conditioned rabbits the villous hypertrophy and round cell infiltration became more pronounced and in several instances

large edematous villi were seen to contain abundant aggregations of lymphocytes and plasma cells. Moreover, the necrosis of the cartilage became more extensive and in some animals was seen to replace most of the articular surface.

No histologic alterations were observed in other organs except for the hamstring muscles which showed focal infiltration with lymphocytes and plasma cells, replacement of muscular elements by connective tissue and, in the early stages, deposition of lime salt. Such changes were found as early as 10 days and as late as 6 months following treatment.

Recovery from arthritis occurred frequently in the conditioned groups in which the joint was injected with NDS. This, however, was not the case when the arthritis was produced by DARKS in either normal or NaCl conditioned animals. Recoveries from DARKS induced arthritis were observed only in animals preconditioned with KCl, in the intradermally preimmunized and in a few nonconditioned rabbits in which NDS was simultaneously injected into the contralateral joint.

The preimmunized group developed precipitins against duck serum as early as 48 hours following the i.a. injection of either DARKS or NDS. The intensity of the precipitin reaction reached 5+ on the eighth day, followed by a rapid decline during the third week. All other experimental groups showed circulating anti-duck serum precipitins appearing on the seventh day following the first i.a. injection of duck serum. Such circulating precipitins reacted in equal degree with DARKS, with NDS, as well as with their isolated globulins. During the early phase the precipitate was significantly higher in the capillary tubes containing the serum of conditioned as compared to nonconditioned groups regardless of whether they have been treated with DARKS or with NDS. At the end of the fourth week, however, the serum of nonconditioned animals gave a precipitin reaction which was roughly equivalent to that of the conditioned rabbits. By that time the conditioned groups displayed a fully developed arthritis. From the fifth week on, both conditioned and control groups showed a progressive decline in the precipitin reaction occurring between serum and duck globulins. The decrease was more precipitous in animals developing arthritis, *i.e.*, conditioned, than in those showing no symptoms, *i.e.*, control. In the preimmunized and in the conditioned groups the peak of the arthritis coincided with the peak of the precipitin reaction.

Discussion. These studies demonstrate that a high salt intake accelerates the development and accentuates the severity of arthritis produced by i.a. injections of DARKS in rabbits. The mechanism of this conditioning effect is not entirely clear. It did not depend on high sodium intake since similar results were obtained with potassium chloride. Moreover, in such conditioned animals not only DARKS but normal duck, calf or horse serum also produced arthritis without preliminary immunization. This could not be obtained in nonconditioned controls.

The study of the precipitin reaction occurring in capillary tubes between rabbit sera and isolated duck globulins suggested that salt conditioned animals may develop higher anti-duck antibody titers than nonconditioned controls. Quantitative measurements of antibody nitrogen, now in progress, will help to determine whether or not the antibody formation is enhanced and whether such enhancement could account for the increased severity of the arthritis in conditioned as compared to control animals. All the above varieties of accelerated as well as delayed experimental arthritis had 3 features in common: 1) circulating anti-duck antibodies always preceded development of arthritis, 2) morphologic alterations remained confined to the injected joint and the surrounding hamstring muscles and 3) no pathogenic agent could be demonstrated in exudate or in tissues. In instances in which the injected duck serum was accidentally contaminated, an acute pyogenic arthritis preceded by several days the development of circulating antibodies.

Summary. 1) Normal rabbits injected intra-articularly (i.a.) with 0.5 ml Duck Anti-Rabbit Kidney Serum (DARKS) twice weekly for 6 weeks developed a progressive monoarthritis following a latent period averaging *10 weeks*. Control rabbits treated simi-

larly with Normal Duck Serum (NDS) failed to develop arthritis. 2) Rabbits with a remote history of immunization against duck serum developed an accelerated arthritis within *one week* following i.a. injections of either DARKS or NDS. 3) Nonimmunized animals preconditioned with isotonic NaCl or KCl given instead of drinking water, developed accelerated arthritis within 2 to 4 weeks following i.a. injections not only of DARKS but of normal duck, calf or horse serum as well. 4) Circulating anti-duck serum precipitins always preceded development of arthritis induced by i.a. injections of duck serum. 5) The arthritic changes consisted of villous hypertrophy and infiltration of the synovium with lymphocytes and plasma cells. This was followed by fibrinoid necrosis of the synovium and articular cartilage and by formation in the joint cavity of an exudate of proteinaceous material, round cells and polymorphonuclear leukocytes.

The authors wish to acknowledge the assistance of Dr. Paul Brancato. They are grateful to Mr. Herbert A. Fischler for preparing the photographs and to Mrs. Renee Nakrinsky for typing the manuscript.

1. Teodoru, C. V., Volk, B. W., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 274.

2. Teodoru, C. V., *Fed. Proc.*, 1961, v20, part I.

3. Brancato, P., Teodoru, C. V., Volk, B. W., *ibid.*, 1961, v20, part I.

4. Teodoru, C. V., Saifer, A., Frankel, H., *Am. J. Physiol.*, 1959, v196, 457.

5. Mellors, R. C., *Analytical Cytology. Methods for Studying Cellular Form and Function.* 2nd Edition. McGraw-Hill Book Co., Inc., New York, 1959.

Received March 12, 1962. P.S.E.B.M., 1962, v110.

Effect of Phenylephrine on Responses of the Rabbit Aortic Strip to Isoproterenol.* (27552)

GERTRUDE D. MAENGWYN-DAVIES
(With the technical assistance of Barbara Stuelcken)
Department of Pharmacology, Georgetown University, Medical Center, Washington, D.C.

In the atropinized dog and cat the vasodepressor action of isoproterenol could be converted to a vasopressor effect by infusion of large amounts of sympathomimetic amines (1). Phenylephrine, one of the most potent amines studied, induced vasopressor responses as well as an increase of peripheral resistance and contractions of the nictitating membrane to isoproterenol. The responses to epinephrine remained essentially unchanged(2). *Pretreatment* with large doses of dichloroisoproterenol (DCI) did not alter isoproterenol vasomotor reversal by phenylephrine. Therefore, this phenomenon was attributed in part to a "sensitization" of contractile (alpha, 3) receptors.

Levy and Ahlquist(4) found that *pretreatment* by the alpha receptor blocking agent, dibenzchlorethylamine, inhibited isoproterenol vasomotor reversal and that DCI, administered after isoproterenol vasomotor reversal had been established, markedly reduced the pressor responses. This latter observation was further corroborated and extended in our laboratory. The original height of the pressor responses to isoproterenol could be reestablished by small additional doses of phenylephrine, administered during the duration of the DCI-blockade(5).

Isoproterenol vasomotor reversal by vasopressin was investigated by Nash, Boyajy and Manley(6). Following vasopressin, the increase in femoral arterial blood flow produced by isoproterenol was significantly reduced but not reversed. Blood pressure responses to epinephrine were significantly augmented and the effect of epinephrine on femoral arterial blood flow was reversed. These authors, therefore, concluded that "the reversal of isoproterenol and the potentiation of epinephrine seen in these studies can be explained on the basis of selective blockade of peripheral vaso-

*Supported by grant from Nat. Inst. of Neurol. Dis. and Blindness, U.S.P.H.S.