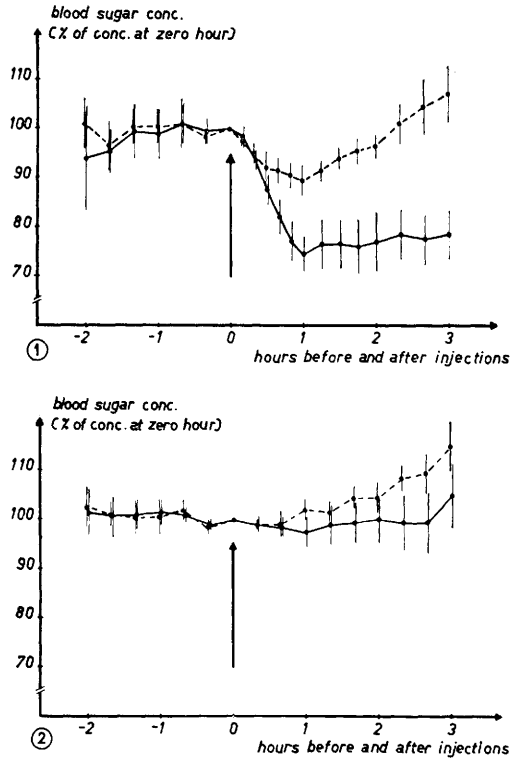

FIG. 1. Blood sugar response of eviscerated cats to insulin 0.01 unit/kg i.v. (dotted curve—avg of 7 animals) and to tolbutamide 100 mg/kg + insulin 0.01 unit/kg i.v. (solid curve—avg of 6 animals). Injections at the arrow. S.E. of mean indicated by vertical lines.

FIG. 2. Course of blood sugar conc. after inj. of saline (dotted curve—avg of 7 animals) and tolbutamide 100 mg/kg i.v. (solid curve—avg of 7 animals). Injections at the arrow. S.E. of mean indicated by vertical lines.

on cats pancreatectomized 2 days before evisceration.

Several investigators(2,3,4,10) including the author(5) have reported that tolbutamide is without effect in eviscerated animals. In some of these works, including the author's, lack of control experiments may have concealed a possible effect of the tolbutamide, as the constant glucose uptake in eviscerated animals injected with tolbutamide gives the impression that tolbutamide has no effect.

The results reported here are in accordance with the findings by Houssay *et al.*(3) of a hypoglycemic effect of tolbutamide in eviscerated dogs continually infused with glucose and insulin.

Summary. An insulin potentiating action of tolbutamide in eviscerated, nephrectomized cats is reported.

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Delayed Monoarthritis in Rabbits Induced by Means of Avian Nephrotoxic Serum.* (26081)

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In a series of experiments in which Duck Anti-Rabbit Kidney Serum (DARKS) was injected into rabbits by various routes other than intravenous, the specific affinity of the serum for kidney tissue appeared doubtful. Based on a pathogenetic concept suggested by these experiments, small amounts of DARKS were injected repeatedly into the stifle of rab-

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bits in the attempt to provoke an arthritis of immunogenic origin. This study presents preliminary experiments in which animals thus treated developed a delayed monoarthritis at the site of injected joint.

Materials and methods. The study was carried out on 14 New Zealand white rabbits of either sex, weighing between 3 and 4 kg, divided into 4 groups.

1) Four animals received Duck Anti-Rabbit Kidney Serum (DARKS) in the right stifle (knee joint). 2) Four other animals received DARKS in the right stifle and Normal Duck Serum (NDS) in the left. 3) Two rabbits received NDS in the right stifle. 4) Four animals were pre-immunized with 2 injections of 0.1 and 0.4 ml NDS given intradermally at 2 day intervals. Six months later one of them received 0.3 ml DARKS, another 0.3 ml NDS, in the right stifle. The remaining 2 animals were treated similarly except that the intra-articular injection was given after a delay of another 3 months.

Intra-articular injections with either NDS or DARKS were given through the anterior interline of the knee joint twice weekly for 6 weeks in amounts of 0.1 and 0.3 ml for the first week and 0.5 ml for the remaining 5 weeks.

DARKS was obtained from ducks immunized with a 10% suspension of blood-free, homogenated rabbit kidneys following the procedure previously described(1). NDS was obtained from normal duck blood submitted to the same processing routine as the DARKS. All rabbits were periodically bled. The serum was frozen and stored. Renal damage was ruled out by urinalysis, by routine blood tests previously described(1) and by histologic examination of the kidneys.

Careful clinical and roentgenologic evaluations of the knee joints were periodically performed on all animals. The severity of the arthritis during the acute stage was expressed as 1+ to 5+ the highest number indicating severe clinical symptoms.

Antibody titers of the various samples of rabbit serum were determined semi-quantitatively by the precipitin reaction between rabbit and duck sera in capillary tubes incubated at 37°C for 4 hours and placed afterwards into the refrigerator overnight. Intensity of the reaction was measured by the height of the precipitated columns and expressed as 1+ to 5+. A precipitin reaction filling approximately 50% of the capillary tube was estimated as 5+.

The rabbits were sacrificed by overdosage with barbiturates. The kidneys and both knee joints were immediately fixed in 10% buffered formalin solution for histologic studies. After decalcification and paraffin embedding, sections were stained with hematoxylin and eosin and a modification of the Masson technic(2).

Results. Four of the 8 rabbits which had no previous experience with duck serum, developed a severe monoarthritis during the 6 week period following the last intra-articular injection of DARKS. The remaining 4 animals also developed arthritis but only 2 weeks after a "booster" injection of 0.5 cc DARKS. This injection was given intra-articularly, 9 weeks after termination of initial treatment.

Animals injected into each stifle, *i.e.*, DARKS into the right and NDS into the left, developed a monoarthritis only in the stifle injected with DARKS (Fig. 1).

The 4 animals which had been injected with NDS intradermally several months previously, developed an *accelerated* arthritis within one week following the first intra-articular injection of either DARKS or NDS.

The first clinical sign of arthritis was swelling. It was obviously associated with pain and marked functional impairment as revealed by the reactions and posture of the animals. Crepitus and limitation of passive luxation of the rotula were the most frequent additional findings. The acute stage was usually followed by a chronic, progressive evolution in which hyperostosis, deformation of the condyles and functional limitations were the main clinical features. Serial roentgenographic studies taken during development of arthritis showed 3 main stages of evolution: a) Swelling of the soft tissues with bulging of joint capsule (Fig. 2). b) disappearance of the cartilage with narrowing of condyles and hyperostosis (Fig. 3), eventually leading to c) osteosclerosis (Fig. 4).

The gross pathologic findings varied with

the stage of arthritis. Dullness of the cartilage, bone lipping at periphery of the cartilage, thickening of the capsule, hyperostosis with new bone formation in the soft tissues were the common gross autopsy findings.

Microscopic examination of the decalcified sections of joints showed diffuse infiltration of the synovia and cartilage by segmented leukocytes and ulceration of the cartilage leading to exposure of the underlying bone.

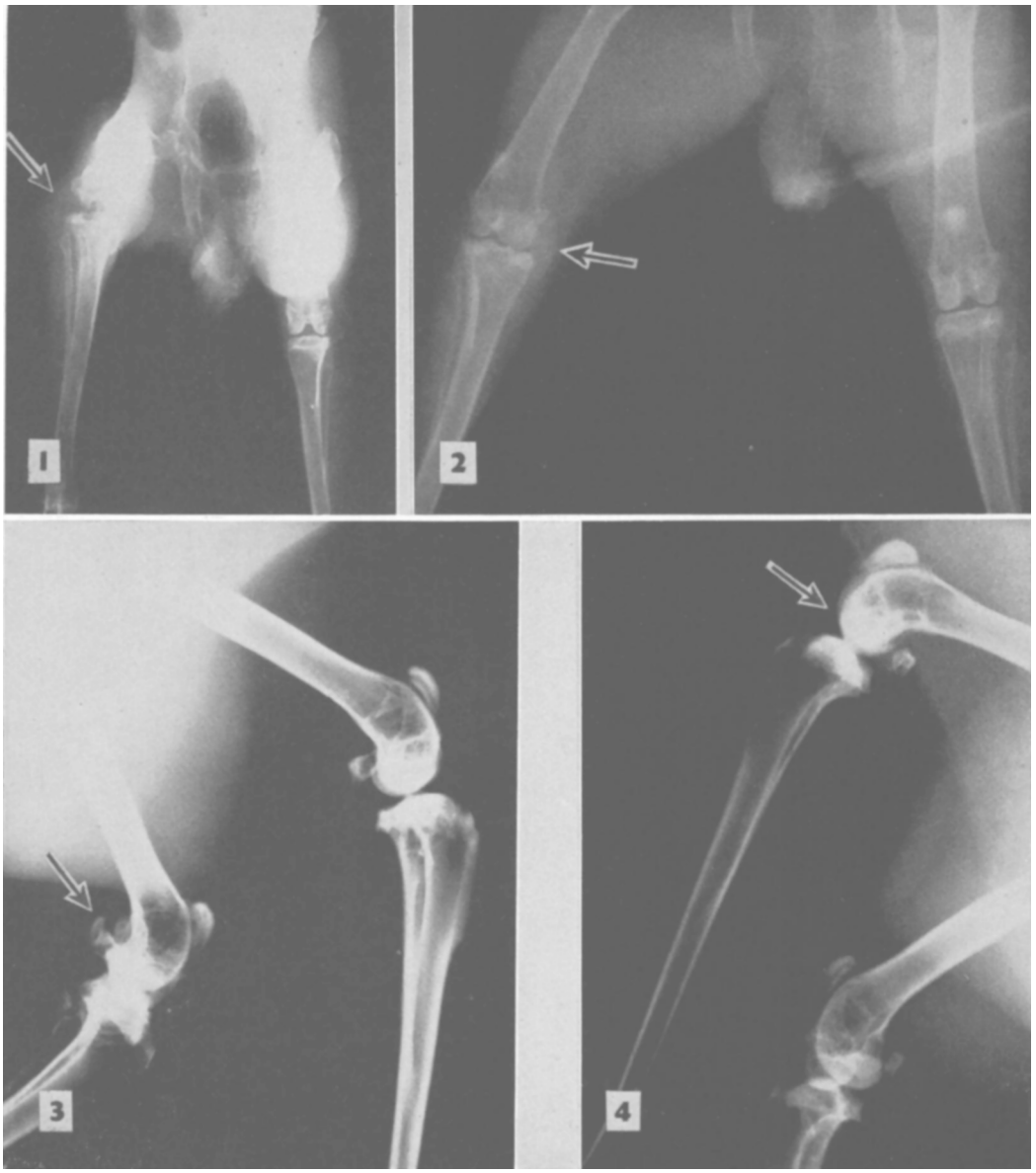

FIG. 1. Roentgenogram taken 4 wk after intra-articular treatment with DARKS, (right stifle), and NDS, (left stifle). Arrow points the severe arthritis of the joint receiving DARKS. The left stifle treated with NDS appears unchanged.

FIG. 2. Roentgenogram taken 6 wk after last intra-articular inj. with DARKS. There is bulging of joint capsule and swelling of soft tissues (arrow) representing first stage in development of arthritis.

FIG. 3. Same animal as in Fig. 2, 12 wk later. Arrow points at hyperostosis as second stage of arthritis.

FIG. 4. Same animal as in Fig. 3, 4 wk later. Arrow points at osteosclerosis as final stage.

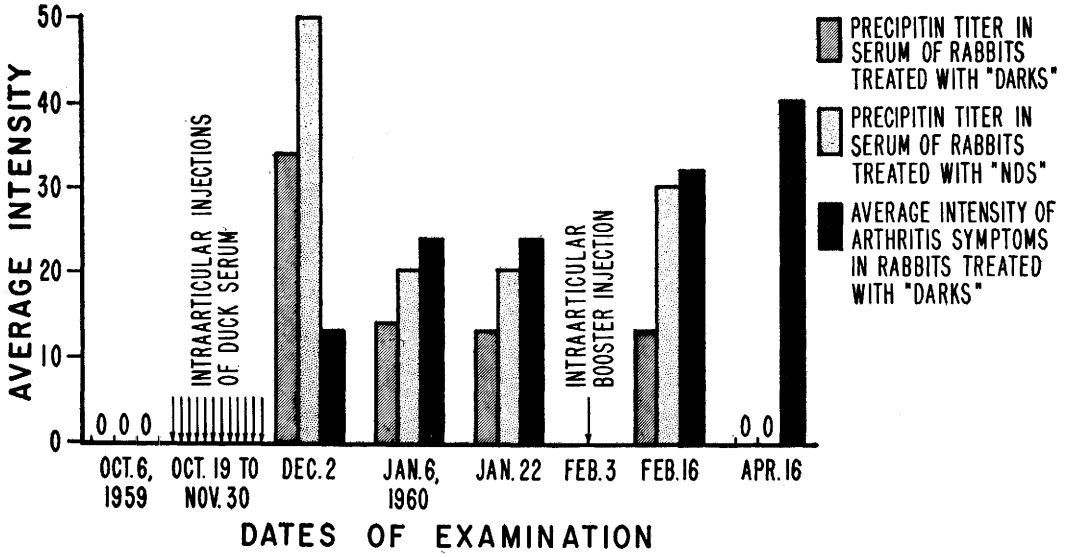

FIG. 5. Avg anti-duck precipitin titer in blood of rabbits treated with either DARKS or NDS given intra-articularly twice weekly for 6 wk. Avg intensity of arthritic symptoms increases as serum precipitin titer decreases in rabbits treated with DARKS. Avg intensity of arthritic symptoms as well as of precipitin reactions are computed according to the conventional formula:

$$\text{Avg intensity} = \frac{\Sigma\,(n+) \times 10}{N}$$

$\Sigma\,(n+)$ = sum of (+) expressing severity of symptoms or intensity of precipitin reaction for individual animals of each group. N = No. of animals in each group.

The joint capsule was edematous, congested and also infiltrated with polymorphonuclear leukocytes. The uninjected contralateral joint which served as control appeared normal. The joints which received NDS also failed to develop arthritic changes within 6 months despite intra-articular booster injections. The accelerated arthritis of the pre-immunized animals did not follow the evolutionary pattern of the delayed arthritis but showed a tendency for recovery after the acute period.

The serum of rabbits which had no previous experience with duck serum showed no precipitin reaction with the latter before intra-articular injection. At the end of intra-articular treatment, *i.e.*, shortly before development of arthritis, all serum samples showed intensive precipitin reaction with either DARKS or NDS. In the animals treated with DARKS the precipitin titer diminished progressively and finally disappeared as the arthritis developed (Fig. 5). Control animals injected with NDS only did not develop arthritis and maintained the anti-duck precipitin titer in serum at higher levels and for a longer period than those receiving DARKS (Fig. 5). A direct relationship seems to exist between development of arthritis and disappearance of specific antibodies from the circulating blood.

Discussion. These experiments strongly suggest that the monoarthritis produced by DARKS is of immuno-allergic nature. This is supported by several facts: 1) Arthritis is delayed by at least 8 weeks after the first, and 2 weeks after the last intra-articular injection. 2) It is accelerated when intra-articular treatment is performed on animals pre-immunized with duck serum. 3) Development of arthritis coincides with a sudden diminution or disappearance of the circulating anti-duck antibodies, suggesting their exhaustion at site of affected joint.

A superimposed infection as a cause of arthritis at the DARKS-treated joint, although possible, is not probable. All batches of nephrotoxic serum produced arthritis, while normal duck sera given simultaneously into the contralateral joint produced no

changes. The long delay between intra-articular treatment and development of arthritis also tends to eliminate the possibility of superinfection as main or contributory factor. Furthermore, PPLO have been ruled out by repeated negative cultures.

Despite the fact that both DARKS and NDS produced similar titers in the serum of the host rabbit, only the former was able to produce joint alterations when given intra-articularly, skin necrosis when given intradermally, and kidney lesions when given intravenously. This suggests that DARKS contains antibodies reacting with an element, possibly of mesenchymal origin, common to the 3 different organs, joint, skin and kidney. According to Izumi(3) and Chikamitsu(4), the active principle of the nephrotoxic sera responsible for the damage to the skin as well as to the kidneys are antibodies against mesenchymal elements of the vascular system. The elective, almost exclusive, involvment of the renal vascular system when nephrotoxic serum is given intravenously is due, according to Izumi, to the specific functional and structural character of the kidney which, besides having one of the richest vascular supplies in the body, is capable of concentrating, by ultrafiltration, the various components of the circulating blood(3). Chikamitsu also claims that nephrotoxic sera do not contain antibodies against kidney tissue but against the vascular system in general. His conclusion is based on the fact that when duck anti-rabbit pneumotoxic serum is injected intravenously into rabbits, the animals do not develop pulmonary damage but a pure Masugi nephritis(4). The anti-lung or pneumotoxic serum is, allegedly, a pure anti-vascular serum because of the rich capillary system of the lung.

Studies are in progress, using the fluorescent antibody technic, as to whether the arthritis provoked by the nephrotoxic serum in rabbits is initiated by vascular damage or whether the duck protein becomes attached to other cellular elements of the joint.

Summary. 1. Normal rabbits injected with 0.5 ml of duck anti-rabbit kidney serum (DARKS) in the stifle, twice weekly for 6 weeks, developed a *delayed* monoarthritis starting 2 to 4 months following first intra-articular injection. 2. Control animals injected with a similar dose of normal duck serum (NDS) failed to develop arthritis within a 6 months observation period. 3. The intra-articular injections of either NDS or DARKS produced high serum antibody titers precipitating both, NDS and DARKS prior to development of the arthritis. The titer disappeared as the arthritis developed in rabbits treated with DARKS but persisted in the NDS-treated control animals. 4. When either, DARKS or NDS, was injected into the joint of rabbits previously immunized with NDS, an accelerated arthritis developed within one week. 5. The main clinical symptoms at site of the affected joint consisted of pain, swelling, crepitus, limitation of active and passive movements with subsequent functional impairment. 6. Histologic studies revealed exudate in the joint cavity consisting predominantly of PMN's and infiltration of synovia and cartilage with segmented leukocytes as well as erosion of the cartilage leading to exposure of the underlying bone. 7. The clinico-pathologic findings were paralleled roentgenologically by swelling of the soft tissues, bulging of the articular capsule, hyperostosis, disappearance of the cartilage, narrowing of the condyles, resulting in osteosclerosis at the end of 6 months.

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