mg/100 g bw induces a chronic state of diabetes(4).

When alloxan was injected, feed consumption dropped for a period of 3 days to 67% of control intake but then increased to above normal by the 6th day. During this period 8 rats died. It was thought that these rats might be animals in the lower range of feed consumption. However, a comparison of mean feed consumption of the survivors with those which died did not suggest that their slightly lower normal feed consumption played a role in their death.

The 17 surviving rats continued to increase in feed consumption to a mean 36% above their control period during the 7th to 12th days after alloxan injection and by 56% during days 13 to 18. Since the normal control group showed a slight decline in feed consumption/100 g bw whereas these rats increased in feed consumption, it seemed preferable to compare the change on the basis of the feed consumption of the experimental group during the preliminary control period.

Summary. A group of 25 mature female Sprague-Dawley-Rolfsmeyer rats weighing a mean of 223 g consumed a mean of 5.88 g/100 g bw of Purina Lab Chow daily during a 2-week control period. When alloxan was injected intraperitoneally at a level of 15 mg/100 g bw to induce chronic diabetes, feed consumption declined to a level 67% below the control period, then began to increase. Eight rats died during the first week following treatment. The 17 surviving rats increased feed consumption 36% during the 7th to 12th days and 56% during the 13th to 18th days. These data show that induction of chronic diabetes by alloxan increases feed consumption by over 50%.

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Production of Aortic Thrombosis in Rabbits. (30194)

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Arterial thrombosis as a complication of atherosclerosis has only rarely been produced in experimental animals. Most of the successful attempts involved physical damage to the arterial wall(1,2). In recent years some investigators utilized differences in electrical potentials (injury currents) to produce aggregation of cellular elements on the vascular intima leading to thrombosis in otherwise normal, non-atherosclerotic vessels(3). This was done by introducing electrodes *in vivo* (properly oriented as to polarity) into the intima of the vessel in dogs and rabbits, or by surrounding a segment of canine aortic wall(4), or the meso-appendix of the rat with excised autologous skeletal muscle. Whether the change in polarity between intima and adventitia is the only, or most important, cause of thrombus formation or whether other alterations are equally important has not been clearly established.

The present experiments were undertaken to determine whether excised skeletal muscle, wrapped around the abdominal aorta of normal rabbits can consistently produce arterial thrombosis.

Material and methods. Three series of experiments were carried out using a total of 103 rabbits. The abdomen was opened under sodium pentothal anesthesia. Approximately 1 cm of the abdominal aorta between the

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TABLE I. Summary of Results.

Groups	Total No. of rabbits in group	Sham-operated controls			Muscle implant		
		Total	No. with thrombus	% with thrombi	Total	No. with thrombus	% with thrombi
Series 1:							
Young rabbits (approximately 3 mo old)	23	9	0	0	14	0	0
Series 2:							
Old rabbits (11 mo or more)	42	14	0	0	28	14	50.0
Series 3:							
Young rabbits pretreated with Vit. D* (approximately 3 mo old)	38	14	1	7.1	24	13	54.2

* Vit. D dosage: 1500 μ/kg B.W. for 3-6 days pre-operatively.

renal arteries and the iliac bifurcation was dissected free from surrounding tissue. In the experimental animals a small strip of autologous pectoral muscle, with multiple cuts made by a scalpel, was placed around the prepared aortic segment, the cutup side lying against the aortic adventitia. The two ends of the strip of muscle placed around the aorta were sutured to each other to keep it in place. Control animals were treated in an identical manner except that there was no implantation of muscle ("sham" operated). Series 1 consisted of young rabbits approximately 3 months old. In series 2, older rabbits were used (at least 11 months old). The third series consisted of young animals as in series 1, but the animals were pretreated with large doses of vit. D (1500 units/kg body weight orally for 3-6 days preoperatively). Vitamin D was chosen because it has been shown by several investigators(5,6) to produce aortic calcification, particularly in the media around the elastic lamellae, as well as other alterations in ground substance like those seen in old animals. Large doses of vit. D administered for 4 consecutive days were shown by Constantinides to be adequate to produce these changes(7). All animals were killed 24 hours after surgery by an overdose of sodium pentothal.

Results. The results are given in Table I. Aortic thrombosis was produced in 50% of the older, normal rabbits. Young rabbits, however, were resistant to the production of thrombosis. This resistance was overcome by a short pretreatment period with large doses of vit. D. No thrombosis occurred in the "sham" operated controls.

Fig. 1 to 4 show several stages of thrombi. The thrombus varied from a simple aggregate of red cells and platelets (Fig. 1) to more advanced stages with beginning organization (Fig. 2). Occasionally, the whole aortic wall showed some round-cell infiltration (arteritis) as in Fig. 3. Dissection of the aortic wall at the site of thrombosis was sometimes observed (Fig. 4). Conventional light microscopy, even with special staining procedures, (Van Kossa), failed to reveal any calcium in the aortae of either the vit. D treated or the old animals.

Discussion and conclusion. Implantation of autologous degenerating skeletal muscle is capable of inducing arterial thrombosis in the rabbit aorta. Injury currents were not measured in our experiments, but Sawyer *et al,* who originated this method, have conclusively shown that they exist(4). If injury currents and changes in electrical potential across the vascular wall are partially responsible for thrombosis, they do not seem to be the only important variable. Our results show clearly that the age of the animal is significant in determining whether or not thrombus formation occurs. This may be due to local changes in the aortic wall or possibly to other biological changes accompanying aging. It has been established that with age ground substance changes of aortic wall occur regularly. An increase in hydroxyproline and hexosamine has been shown by Bertelson to occur with age and these changes

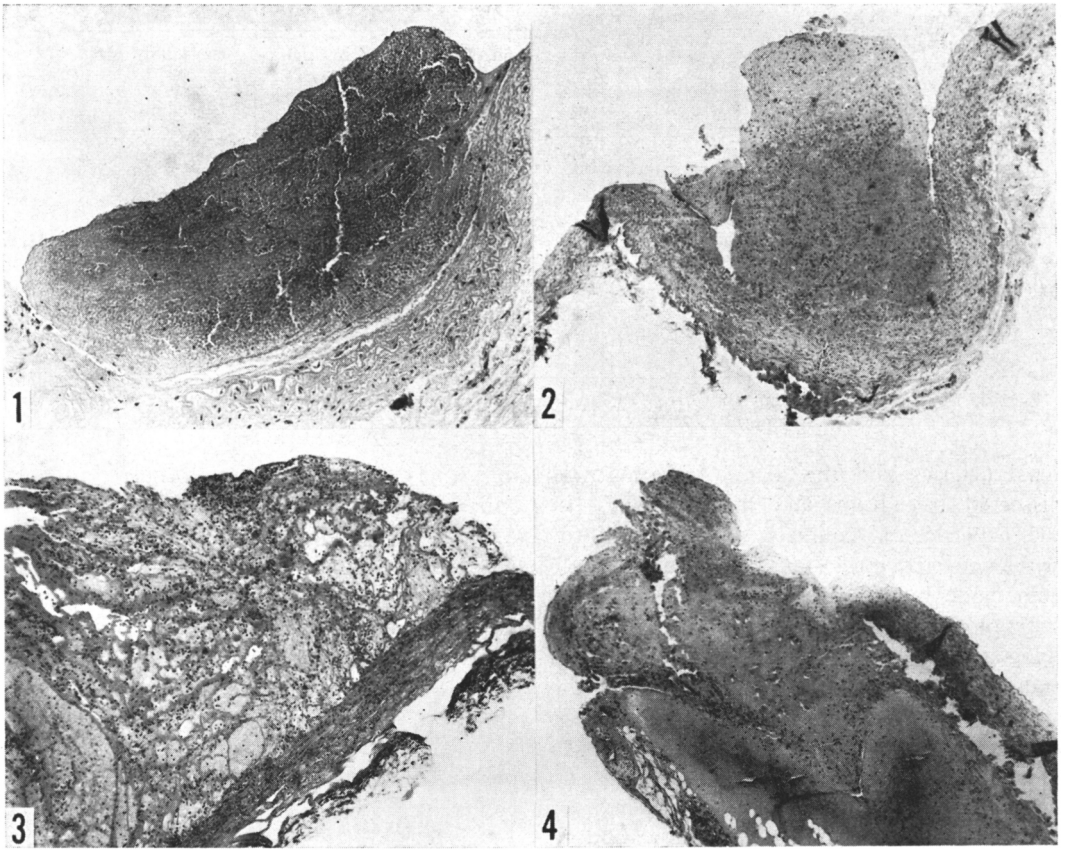

FIG. 1. Fresh thrombus in abdominal aorta of a rabbit. H & E, × 90.
FIG. 2. Large white thrombus with beginning organization in aorta of a rabbit. H & E, × 36.
FIG. 3. Large organizing thrombus with large amount of fibrin and round-cell infiltration in the underlying vascular wall. H & E, × 36.
FIG. 4. White thrombus over a dissected aortic wall. H & E, × 36.

are considered by him to be the first sign of aortic degeneration(8). The same changes have been shown to occur in hypervitaminosis D after 8 days(9). In the rat, after 4 days, changes in aortic hexosamine were seen (6). The changes with age and vit. D may also affect the calcium (or calcium ion) content of the vessel wall. Therefore, it can be postulated that hypervitaminosis D "ages" the arterial wall of the rabbit and makes it susceptible to thrombus formation.

We have also produced aortic thrombosis by this method in chicks. However, in this species the results were not nearly as consistent as in the rabbit. We are currently investigating the possible cause for these differences.

Summary. Exposure of the abdominal wall to injured skeletal muscle produces thrombosis in 50% of old rabbits and young rabbits pretreated with large doses of vit. D. Young untreated rabbits are resistant to thrombus formation by this method.

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Separation of Neurotoxin and Hemolysin of *Clostridium tetani*. (30195)

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The most prominent manifestations of tetanus are due to a neurotoxin produced by *Clostridium tetani*. Little attention has been given to the possible role of other toxic substances of this bacterium in the pathogenesis of the clinical disease or the need for their presence in prophylactic antigens or their antibodies in therapeutic serum. One other substance to be considered is the hemolysin, tetanolysin.

Shortly after Faber(1) demonstrated the existence of a neurotoxin in filtrates of cultures of *C. tetani*, Ehrlich(2) observed the presence of a hemolysin in cultures of this organism. Characteristics of these substances have been reviewed by Fleming(3). The lysin is set free during the period of active growth of the culture, while most of the toxin liberation occurs after the bacterial multiplication has ceased; the lysin may be absorbed to red blood cells at 0°C but the neurotoxin lacks this property; the hemolysin is more heat and oxygen labile than the neurotoxin; both the lysin and the neurotoxin induce specific antitoxins. Imbriano(4) stated that the hemolysis is the result of esterase or protease activity. The tetanolysin is necrotizing(5), cardiotoxic(6), and is closely related serologically to other oxygen-labile hemolysins, *e.g.*, pneumolysin, streptolysin O, and the theta toxin of *Clostridium perfringens*(6).

Because of the *in vitro* inhibitory activity of normal sera(7) and sterols(4,6) on tetanolysin, *in vivo* effects have been discounted. However, the possibility remains that the hemolysin is contributory to the anemias(8) and the cardiac complications(9) in patients with tetanus.

Previous laboratory studies(3,4,7) of the hemolysin have been made using crude culture filtrates or ammonium sulfate precipitates from filtrates, each of which, no doubt, contains neurotoxin. The activities and properties of the hemolysin could be defined more accurately if it could be separated from the neurotoxin. This paper reports a high degree of separation of the two toxins by use of gel filtration through Sephadex G-100.

Materials and methods. Crude culture filtrates. Five ml of a 48-hour-old culture of the Massachusetts C_2 strain of *C. tetani* in thioglycollate broth were seeded into a dialysis bag (Visking) containing 250 ml of saline immersed in 2500 ml of a protein-free medium(10). The culture was incubated at 37°C for 7 days. The contents of the bag were centrifuged in the cold at 2000 RCF for one hour and the supernatant filtered through a Millipore® filter (HA, 0.45 u). The filtrate was stored at 5°C and then brought to dryness by freeze-drying. Restoration to one-tenth the original volume was made with phosphate buffer, pH 7.0, 0.025 M. The concentrate used to obtain the results given here contained approximately 10^6 mouse MLD and 6×10^5 hemolytic units per ml.

Gel filtration. The chromatographic procedure of gel filtration through Sephadex G-100 (Pharmacia)(11) was used for separation of the hemolysin and the neurotoxin. The Sephadex G-100 was allowed to swell in phosphate buffer, pH 7.0, 0.025 M for 48 hours. After repeated washings and decan-