ACUTE SELENIUM TOXICOSIS IN THE BABY PIG

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ABSTRACT

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Sodium selenite was fed to 3-week-old pigs to determine the symptoms, hematology and gross and microscopic lesions. One lot was fed a basal ration while in 5 additional lots the basal ration was supplemented with 0.5, 1.0, 2.0, 4.0, and 8.0 mg. selenium per lb. body weight, respectively. The two higher levels were lethal in approximately 48 hours, while the lower dosages were progressively less toxic.

Signs of anorexia, vomiting, diarrhea, hypothermia, dyspnea, and incoordination were noted. Hemoconcentration was present in the acutely affected animals while serum glutamic oxalacetic transaminase values were increased approximately 10 to 20 fold.

The most severe lesions were noted in the groups fed the largest amounts of selenium. Gross tissue changes included: fatty livers, congested blood vessels; congestive catarrhal gastroenteritis, often with gastric ecchymoses and ulcers; occasional epicardial hemorrhages; and cyanosis.

Histopathological manifestations were most severe in the liver, kidneys, and gastrointestinal tract. Fatty metamorphosis, centrolobular necrosis, and congestion were noted in the liver. Degeneration and fatty changes of the renal tubules plus varying degrees of congestion were present in the kidneys. The most evident gastrointestinal lesions included ulceration, exfoliation, and erosion of mucosal epithelium and congestion, hemorrhages and leukocytic infiltration.

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By

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INTRODUCTION

The role of selenium in animal and human health has become of major importance in recent years. It has been known for many years to be a toxic substance and recent research has demonstrated it to be a required nutrient for most species of livestock. In addition, contamination of foodstuffs such as cereals is of public health concern.

The toxicity of selenium has long been a problem which, despite much research, has defied complete solution. The effects of various toxic doses on laboratory animals has been recorded by many workers. Reports on toxic levels for farm animals are less numerous. Selenosis in sheep, horses, cattle, and swine has been studied but detailed histopathological findings were often limited or lacking entirely.

Recently reports have been published demonstrating that selenium is a growth factor for most species of livestock. In New Zealand, the administration of selenium to lambs suffering from "ill thrift" reduced mortality and improved gains (Drake et al., 1960) while Jolly (1960) reported improved weight gains in calves fed rations supplemented with selenium. It has been shown to reduce the incidence of muscular dystrophy in lambs when fed to pregnant ewes (Muth et al., 1959). Subcutaneous injections have also proved beneficial in reducing the incidence of muscular dystrophy in lambs (Lagace, 1961).

The public health aspects of selenium poisoning should not be minimized. Smith and co-workers (1936) stated that bad teeth, skin disturbances, chronic arthritis, and cardio-renal problems were found in 111 families residing on seleniferous Dakota soils. Ill health was also prevalent in workers engaged in the processing of selenium, according to Dudley (1936a).

The recent findings that selenium is a nutritional as well as a toxic factor emphasizes the need for additional research, especially on the histopathologic aspects of acute toxicosis.

LITERATURE REVIEW

The literature on selenium is so voluminous that only the references pertinent to its toxicity and role in nutrition in animals is reviewed in detail.

According to Moxon and Rhian (1943) the first authentic record of selenium poisoning ("alkali disease") in livestock was written in 1856 by Madison, an army surgeon stationed at Fort Randall, South Dakota. He reported a fatal disease of cavalry horses. The roles of geological formations, vegetation, and weather in selenium toxicity were extensively reviewed by Trelease and Beath (1949).

Selenium toxicosis has been divided into acute and chronic types. Highly toxic or lethal doses produce the acute type, while small repeated doses over long periods of time produce chronic symptoms.

Acute Selenosis

The minimum lethal dose of selenium for acute toxicosis in the common laboratory animals has been discussed by many researchers and much disagreement exists. Franke and Moxon (1936) at the South Dakota Experiment Station reported that intraperitoneal injections of 3.25 to 3.5 mg. of selenite per Kg. of body weight or 5.25 to 5.75 mg. selenate per Kg. body weight was lethal to rats in two days. Smith and co-workers (1940) at the National Institutes of Health stated that the method of administration did not affect the toxicity of selenium. The minimum lethal dose for rats, rabbits, and cats was given as 1.5 to 3.0 mg. per Kg. body weight. This dosage was confirmed on dogs by Anderson and Moxon (1942). However, organic selenium compounds were less toxic than the inorganic, regardless of the route of administration (Moxon *et al.*, 1938).

Lethal doses in farm animals have also been studied, although on a limited scale. Miller and Williams (1940a) found the minimum lethal dose of sodium selenite to be 1.5 mg. per lb. body weight in horses, 4.5 to 5.0 mg. per lb. in cattle, and 6 to 8 mg. per lb. in swine. Kuttler et al. (1961) reported that 80 mg. of sodium selenite per 100 lbs. body weight were lethal and 40 mg. per 100 lbs. body weight were toxic to sheep.

Among laboratory animals the signs of acute toxicosis are essentially identical. Czapek and Weil, Modica, Jones, Smith, Anderson and Moxon are cited by Trelease and Beath (1949) as having described the following: garlicky breath; signs of nervousness, fear and excitation; vomiting and diarrhea; dyspnea followed by opisthotonos and tetanic and clonic muscle spasms; and finally somnolence and death.

Acute signs of toxicosis in South Dakota farm animals were described by Beath and associates (1935b). Manifestations included a characteristic "tucked up" stance with abdominal pain and bloating; diarrhea; elevated temperatures; rapid, weak pulse; labored breathing and pale or cyanotic mucous membranes. Prostration occurred shortly before death.

Descriptions of the gross and microscopic lesions of acute selenium poisoning are extremely limited. Smith (1937a) noted that cats which died in 7 to 11 days from selenium poisoning exhibited fatty degeneration and centrolobular congestion of the liver and fatty degeneration of kidney tubules. Focal hemorrhages and edema of the lungs were the only other lesions observed. Gross and microscopic lesions in 10 sheep were recorded by Rosenfeld and Beath (1946a). These included hemorrhage and congestion of the endocardium, lungs, spleen, kidneys,

liver, gall bladder, pancreas, intestines and omasum. Necrosis and degenerative changes were present in the liver, kidney tubules and gastrointestinal tract. Muscle atrophy was also noted. Miller and Williams (1940a) made almost identical observations in a pig.

Chronic Selenosis

Levels of selenium which produce chronic toxicosis vary widely with diet and species. Doses of 10 ppm to 52 ppm in the diet of rats have been reported by Smith (1939) and Franke and Potter (1935) to produce chronic manifestations. Smith (1940) reported that 14 ppm selenium in the diet of rabbits produced only moderate liver fibrosis, while 3 to 13 ppm were lethal to a dog after 135 days (Rhian and Moxon, 1943). In pigs 24.5 to 392 ppm of sodium selenite in the feed produced death in 10 to 99 days (Miller and Schoening, 1938). Rosenfeld and Beath (1945) at the Wyoming Experiment Station agreed with Smith (1940) that 1.0 to 1.5 mg. of inorganic selenium per Kg. per body weight per day was necessary to produce important pathological changes.

Toxic effects of selenium are not restricted to the host animal. McConnell (1948) reported that in rats 2.5 to 9.3% of the selenium is transferred to the young via the mammary glands and that the selenium found in the milk is entirely in the protein fraction as part of an organic complex. Trelease and Beath (1949) cited the work of Franke and associates (1936) in which as little as 0.01 mg. of selenium injected into incubating hens' eggs caused deformities in the chick. Yet, in spite of this extreme sensitivity of the chick embryo, young and adult chickens are apparently unaffected by toxic grains. Westfall *et al.* (1938) demonstrated that selenium is transmitted through the placenta to the fetus in rats and cats, but no deformities were noted. He

concluded that the extraordinary susceptibility of the chick embryo to selenium is a species characteristic not shared by mammals. However, more recent work by Wahlstrom and Olson (1959) indicated that sows on a ration containing 10 ppm selenium gave birth to an increased number of dead pigs. In addition, conception rate was lowered and the number of services per conception increased. The number of days from weaning to estrus was also increased.

The principle signs of chronic poisoning in laboratory animals are reduced appetite, stunted growth, emaciation, and loss of hair (Trelease and Beath, 1949). Lesions are mainly restricted to the liver. Lillie and Smith (1940) reported that cirrhotic changes were first noted after 5 weeks in rats on a seleniferous diet. After 11 to 15 weeks, increasing fibrosis replaced the proliferative changes in the liver, while the kidneys and other organs remained essentially normal. Hepatic cell adenomas and low grade hepatic cell carcinomas are apparently associated with the cirrhotic changes in the livers of these animals, according to Nelson et al. (1943). Of 53 rats fed 5 to 10 ppm selenide for 18 months, 11 had neoplastic livers, while in a corresponding control group of 350 rats only 4 tumors appeared. Seifter et al. (1946) reported the occurrence of thyroid adenomas in rats fed selenium.

In farm animals selenosis is of two types: a fairly acute, often fatal type called "blind staggers" and a milder, more chronic form called "alkali disease." The signs and lesions of the two syndromes are different (Draize and Beath, 1935). Signs of blind staggers include: aimless wandering, anorexia, impaired vision, and finally abdominal pain, dyspnea and death (Beath et al., 1934). Alkali disease, on the other hand, is characterized by emaciation, loss of hair,

deformation and sloughing of hoofs, and general loss of vitality (Trelease and Beath, 1949). Signs may be delayed for several months (Beath, 1935a).

The lesions of both syndromes have been well documented. There is essential agreement that the morbid changes in blind staggers includes smooth muscle atrophy of the gastrointestinal tract, gall bladder and urinary bladder; congestion of liver and kidneys; areas of focal necrosis in the liver; mucosal degeneration of the stomach and small intestines; and hemorrhages of the epicardium. The lesions of alkali disease are more chronic. Atrophy of the heart, atrophy and cirrhosis of the liver, degeneration of the kidneys, and erosions of the joints are most often noted while petechial hemorrhages of the epicardium and degenerative changes in the gastrointestinal tract are uncommon (Draize and Beath, 1935; Miller and Schoening, 1938; Miller and Williams, 1940b; Moxon, 1937; Rosenfeld and Beath, 1946a; and Schoening, 1936).

Distribution of Selenium in the Tissues

The distribution of selenium in the tissues of the horse, sheep, hog and calf was studied by Dudley (1936b). He reported that in acutely poisoned animals, the liver, kidney, and spleen contained the greatest quantity (4.0 to 25.0 ppm, wet weight) of selenium. Work on dogs by Rhian and Moxon (1943) confirmed Dudley's results. McConnell (1941) found that the liver and kidneys of rats contained the highest levels of selenium two hours after injection of radioselenium.

In chronic toxicosis the distribution of selenium in the body tissues differs somewhat from acute cases. Dudley (1936b) reported that in chronic cases the concentration of selenium was much higher in the kidney than in the liver, while in acute cases the reverse was true.

Kidneys analyzed by Kuttler et al. (1961) contained at least ten times more selenium than the livers, thus further substantiating the results of Dudley. Rosenfeld and Beath (1945) found the largest amounts of selenium in the livers and kidneys of sheep, while the brains and muscles contained only minute amounts. Fat was negative for selenium.

Amounts of selenium in the blood vary widely. In acute cases Dudley (1936b) found the concentration to range between 7 and 27 ppm, whereas in chronic cases the range was 0.2 to 5 ppm. Most of the selenium was present in erythrocytes as part of a protein complex. The serum, plasma and fibrin were negative for selenium. This was generally substantiated in cats by Smith et al. (1937b). McConnell (1941) reported the maximum concentration in the blood 15 minutes after one subcutaneous injection of sodium selenate in rats. The selenium at first appeared more abundantly in the plasma than in the erythrocytes, but this was reversed after 6 hours, apparently because selenium was eliminated from the plasma at a faster rate than it was incorporated into the red cells. Minyard and co-workers (1957) at the South Dakota Experiment Station determined selenium blood values in a large number of steers and concluded that values of more than 2 ppm indicate probable tissue damage; 1 to 2 ppm, possible damage; 0 to 1 ppm, no tissue damage. Blood levels dropped rapidly when selenium was discontinued.

Hematological changes are not restricted to the transportation of selenium, however. Anderson and Moxon (1942) reported marked increases of hemoglobin and packed corpuscle volume in dogs injected subcutaneously with sodium selenite. Blood phosphorus, non-protein nitrogen, calcium, ascorbic acid, and sugar were decreased. Franke and Potter (1935) noted hemoglobin increases up to 25 gm. per 100 ml. blood during the first 9

to 17 days of selenium ingestion in rats. After this time, anemia developed. In-vitro studies by Rosenfeld and Beath (1948) demonstrated that plasma had the ability to reduce selenate to selenite plus a volatile selenium, whereas in erythrocytes no such reduction took place. However, most of the conversion from the selenate to the selenite took place in the liver and the author concluded that toxic reactions are due to the selenite, and that transformation followed this pattern: selenate to selenite to "combined form" to volatile and elemental selenium.

Excretion studies have somewhat clarified the metabolism of selenium in the body. McConnell (1942) reported that in rats 3 to 10% of the original subcutaneous dose of selenium was excreted through the lungs in 24 hours. Gortner and Lewis (1939) and Anderson and Moxon (1941) indicated that 20 to 50% of the ingested selenium is excreted in the feces, a major part of this being unabsorbed selenium. The kidneys excreted 30% of the absorbed selenium, according to work in sheep by Rosenfeld and Beath (1946a). Furthermore, the same authors (1945) found that it takes approximately 60 days for sheep to deplete their tissues of selenium, while cats and rats require only two weeks (Smith et al., 1937b; Anderson and Moxon, 1941). Only small amounts of selenium are found in the urine shortly before death, due mainly to the kidney damage which prevents excretion and results in larger tissue storage (Miller and Williams, 1940b; Rosenfeld and Beath, 1945).

Factors Preventing Toxicity

A variety of rations and supplements have been fed to animals in an effort to prevent or reverse selenium toxicosis. Franke and Potter (1935) demonstrated that alternate feeding of seleniferous corn and

rations devoid of selenium gave growth and food consumption curves of rhythmic decreases and increases in rats. Diets high in protein decreased the toxicity of selenium in rats (Smith, 1939; Gortner, 1940; Lewis et al., 1940). This was confirmed in sheep by Rosenfeld and Beath (1946b). Fels and Cheldelin (1948) noted that the inhibition of yeast growth by selenate was reversed by the addition of methionine. However, Klug et al. (1952) reported that 0.5 to 1% methionine increased weight gain only slightly in rats on a 23 ppm selenium diet, while it did not result in lower selenium content of the livers. He therefore refuted the idea that methionine antagonized or inactivated selenium in vivo. Olson et al. (1958) stated that methionine had its greatest effect at the lower selenium levels. Choline and betaine also gave some protection.

Attempts to cure selenosis by various chemicals have also been made. Moxon et al. (1940) reported that p-bromobenzene increased the excretion of selenium in dogs and steers. This could not be duplicated by Westfall and Smith (1941) in rabbits. The recovery rate of steers poisoned with selenium was not increased by the administration of p-bromobenzene (Minyard et al., 1957). Rosenfeld and Beath (1947) reported that the toxicity of selenium in rats was not altered by the ingestion of increased amounts of ascorbic acid while injections of potassium iodide increased the adverse effects of selenium.

Arsenicals have been used repeatedly for prevention of selenium toxicosis. Rhian and Moxon (1943) reported that 5 ppm sodium arsenite prevented weight loss, anorexia, and anemia in dogs, but did not result in lower selenium content of tissues. The theory that arsenic inhibits absorption of selenium could not be confirmed in further work (Moxon et al., 1945). Organic arsenicals, viz. 0.01% arsenilic acid and 0.005%

3-nitro-4-hydroxyphenylarsonic acid, prevented symptoms and increased the gains in growing pigs fed 7 to 11 ppm sodium selenite in the diet (Wahlstrom et al., 1956). The protective effect is not as pronounced in steers, according to Minyard and associates (1957).

In summary, it is evident from the literature that much more detailed information is needed on the effects of ingestion of selenium by animals. According to Trelease and Beath (1949) many unexplained factors enter into the selenium problem. Accurate minimum dosages of the various forms of selenium for each species of animal are still unknown. No investigator has yet reported on the cause of the final collapse of animals. Likewise the matter of individual tolerances to selenium has not been fully explained.

MATERIALS & METHODS

General Procedures. A litter of 12 Yorkshire pigs was used to study the effects of feeding various levels of sodium selenite. The litter was farrowed on April 6, 1961. Each animal was injected intramuscularly with 2 ml. iron dextran (Haver-Lockhart) when one week old to prevent anemia. The litter was weaned on April 23, 17 days after farrowing.

A period of acclimation to the experimental environment and ration was instituted after weaning. The animals were placed in individual 21- by 36-inch galvanized metal metabolism cages equipped with wire floors and individual feed and water crocks to facilitate accurate measurement of feed and excreta.

On May 1, the animals were divided into six lots of two animals each. Each lot contained a male and a female of approximately equal weight. The control group consisted of two males. Lots 1 through 5 were fed sodium selenite at levels of 0.5, 1.0, 2.0, 4.0 and 8.0 mg./lb. body weight, respectively, and Lot 6, which served as controls, was fed only the basal ration.

Sodium selenite was added to the milk of each animal once daily until death or termination of the experiment. Appropriate amounts of selenite were pipetted from an aqueous solution containing 10 mg. (Baker's grade) sodium selenite (Na_2SeO_3) per ml. In pigs with decreased appetites the amount of milk used to dilute the selenium was reduced to insure complete ingestion of the selenite. The animals were weighed and their feed crocks washed immediately before each daily feeding of selenium.

Ration. Three times daily all animals were fed the basal diet which consisted of 250 ml. homogenized whole milk and free choice of a starter mash. The starter contained the following ingredients: ground corn, 69.5%; soybean oil meal, 28.0%; limestone, 1.00%; trace mineral salt (high in zinc), 0.50%; dicalcium phosphate,0.80%; and a vitamin-antibiotic supplement, 0.25%. The supplement included: Bacitracin, 4.0 Gms./lb.; arsanilic acid, 3.96%; vitamins: A, 600,000 units/lb.; D_2, 300,000 units/lb.; E, 400 I.U./lb.; Riboflavin, 800 mg./lb.; Niacin, 4,000 mg./lb.; B_{12}, 4.0 mg./lb.; choline chloride, 20,000 mg./lb.; Pantothenic acid, 1,600 mg./lb.; and trace minerals. Fresh water was always available.

Signs and Hematological Examinations. Signs were recorded at frequent intervals. Daily temperatures were included.

Blood samples from the anterior vena cava were collected just before the experiment started and four times during the course of the experiment. Leukocyte counts, hemoglobin, packed cell volume (PCV), serum glutamic oxalacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T) determinations were made and recorded. SGO-T and SGP-T activity was determined by the method of the Sigma Research Laboratories (1957). The van den Bergh test for total serum bilirubin was determined on pig E5932 11 days after the experiment started.

Post-mortem Techniques. Necropsy examinations were performed on all animals as soon after death as possible. Animals which could not be examined immediately after death were stored in a refrigerator. Control animals, as well as those that were in moribund condition, were killed by electrocution (120-V A.C.). Tissues for microscopic examination included: liver, gall bladder, kidney, urinary bladder, spleen, esophagus, stomach, small intestine, colon, reproductive tract, thyroid,

adrenals, pancreas, lung, heart, aorta, hoof, tail, proximal part of tibia and skeletal muscle (cross section of semimembranosus mm.).

All tissues except striated muscle were fixed for 24 to 36 hours in Zenker's solution (modified by the exclusion of acetic acid). After fixation, tissues were washed for 24 hours and then stored in 80% ethyl alcohol. Muscle sections were fixed and stored in 10% acetate-buffered formalin. Bone was decalcified with formic acid and sodium citrate.

Sections were cut at 6 microns and stained with Harris' hematoxylin and eosin. In addition, formalin sections of heart, liver, and kidney were stained for fat with Sudan IV. All histopathological procedures used are described in the Manual of Histologic and Special Staining Technics of the Armed Forces Institute of Pathology, Washington, D.C. (1957).

RESULTS

A summary of the general results given in Table I indicates that the toxicosis produced was fairly well correlated with the amount of selenium fed.

Survival times exhibited marked individual variations, expecially those fed the lower dosages of selenium. The two pigs in Lot 5 died in 46 and 50 hours, respectively. The pigs in Lot 4 survived only 54 hours. In Lot 3, one pig died after 3 days, the other after 5 days. Lots 1 and 2, however, were extremely variable. In Lot 2 one pig died after 60 hours while the other survived for 13 days; in Lot 1 only one animal died of selenium poisoning. The other was still alive at the end of the experiment and was placed on a selenium-free diet for 6 days to see if recovery would result. During this time the animal regained its appetite and increased 1.25 lbs. in weight.

Results from a limited number of hematological tests indicated that, of the examinations performed, only hemoglobin, PCV, and SGO-T values were altered. Samples of blood from pigs E5938 and E5940 taken shortly before death indicated a marked increase in hemoglobin and percentage of packed cells. The increase was approximately two-fold over identical tests performed prior to the start of the experiment 3 days previously. However, pigs in Lots 1 and 2 exhibited only moderate increases by the 10th day of the experiment.

SGO-T determinations were also highly elevated. Values from 335 to 870 Sigma-Frankel (S-F) units were obtained from the animals tested. Samples collected on these same animals before feeding selenium ranged between 21 and 32 S-F units.

Table I. Body Weight Changes, Survival Times and Blood Chemical Findings in Pigs Fed Various Amounts of Selenium

Lot and Autopsy Number	Dose (mg/lb body wt/ day)	Av. Total Dose (mg)	Average Starting Wt.(lbs)	Average Final Wt.(lbs)	Av. Wt. Change (lbs.)	Average Survival Time	Hematology (averaged values)					
							SGO-T (S-F Units)***				Hemoglobin	
							Control	5/3	5/9	5/13	Control	Toxicity
Control (E5930) (E5931)	None	None	9.2	13.9	+ 4.7	---	28	-	48*	94	7.9	11.0
1 (E5932) (E5933)	0.5	58	9.4	8.2	- 1.2	12 da.**	25	-	375	485*	7.8	11.8*
2 (E5934) (E5935)	1.0	70	9.0	6.8	- 2.2	8 da.	40	-	495*	870*	8.6	10.0*
3 (E5936) (E5937)	2.0	73	9.4	7.8	- 1.6	5 da.	28	-	-	-	9.7	-
4 (E5938) (E5939)	4.0	77	9.4	7.8	- 1.6	54 hrs.	31	90	-	-	10.3	18.5*
5 (E5940) (E5941)	8.0	146	9.4	7.7	- 1.7	48 hrs.	29	170	-	-	10.5	20.2*

* Only one animal tested
** One animal survived at the termination of the experiment
*** Serum glutamic oxalacetic transaminase values in Sigma-Frankel units

Other tests performed included total leukocyte counts, differential leukocyte counts, SGP-T, and the van den Bergh test. The results of these determinations were not significantly different from normal values.

Signs

Signs and gross lesions observed are summarized in Table II.

In general, the severity of the observed signs varied directly with the dosage of selenium fed. Loss of weight was a pronounced and consistent manifestation in all selenium-fed animals. From 2.5 to 34% of the initial body weight was lost by the treated pigs while the two control animals gained 49 and 59%, respectively (Table I). The most consistent weight loss occurred in the two lots fed the highest amounts of selenium. In less than 3 days these animals lost an average of 17.7% of their body weight. Results were more variable with lower dosages of selenium. Pig E5933, which was fed 0.5 mg. selenite per pound body weight for 12 days made steady weight gains immediately after removal of the selenium from the diet.

Moderate to complete anorexia was noted in all animals. Almost complete anorexia occurred within 24 hours in the three lots fed the highest amounts of selenium. In the two remaining lots, anorexia was delayed several days and was less severe.

Vomiting and diarrhea were pronounced, especially in Lots 4 and 5. In these lots, diarrhea started approximately 24 hours after the initial ingestion of selenium and continued for 6 to 8 hours. The animals appeared uneasy and in acute distress. Their backs were arched and heads lowered. An initial period of severe vomiting was followed by varying periods of retching and diarrhea. The stool was white and pasty in appearance and became progressively more fluid during the course of the experiment.

Table II. Signs and Lesions Resulting from Selenium Ingestion by Pigs (Relative severity expressed in arbitrary fashion as 1+, 2+, 3+, or 4+.)

Animal Number	Lot No.	Days on Se.	Signs							Gross Pathology					
			Anorexia	Vomition	Diarrhea	Temperature Decrease	Dyspnea	Incoordination	Chorea	Fatty Liver	Cyanosis	Epicardial Hemorrhages	Gastric Hemorrhages	Gassy Intestines	Congested Mesenteries
Controls	6	0	-	-	-	-	-	-	-	-	-	-	-	-	-
E5932	1	10	1+	-	-	1+	-	-	-	4+	3+	2+	-	-	1+
33		12	1+	-	-	-	-	-	-	-	-	-	-	-	-
34	2	2	2+	2+	1+	?	-	-	1+	3+	2+	2+	2+	2+	3+
35		12	2+	1+	2+	2+	-	-	-	3+	1+	-	2+	1+	3+
36	3	3	2+	3+	2+	2+	-	1+	-	3+	1+	1+	1+	1+	1+
37		5	3+	3+	2+	1+	-	-	-	2+	2+	-	1+	3+	1+
38	4	2	4+	4+	3+	3+	2+	2+	2+	3+	2+	2+	2+	2+	3+
39		2	4+	4+	4+	3+	2+	2+	-	3+	2+	2+	1+	3+	4+
40	5	2	4+	4+	4+	4+	3+	2+	-	4+	3+	1+	2+	3+	4+
41		2	4+	4+	4+	4+	3+	3+	-	4+	4+	-	2+	3+	4+

These signs were followed by a period of progressive apathy and somnolescence and culminated in coma and death. Gastrointestinal symptoms were milder in Lots 2 and 3, while Lot 1 was not visibly affected. Deaths in Lots 2 and 3 were delayed.

Cyanosis in varying degrees was manifested in all the treated pigs. The snouts, ears, tails, and hoofs were most severely affected, being cold and bluish in appearance. A sharp horizontal line of demarcation separated the dark, purple, distal half of the hoof wall from the more normal appearing proximal half. This phenomenon was noted on all treated animals and was thought to be due to severe congestion and stasis of blood in the large veins of the laminar corium, although this could not be substantiated histologically.

A pronounced drop in body temperatures occurred 12 to 28 hours before death in 8 of the 9 pigs killed with selenium. The body temperatures of the 4 pigs in Lots 4 and 5 averaged 93 F during this time while the range in the less acutely ill animals was 97 to 100.8 F. The lowest reading obtained from the control animals was 101.2 F.

Other signs included dyspnea, which was quite prominent in the most severe cases, and occasional nervous manifestations. The latter were restricted mostly to incoordination and usually occurred before the animal entered the comatose stage. However, two of the animals (E5938 and E5934) suffered from a slight chorea of the head and cervical area. No tetanic of clonic spasms were noted.

Pathology

Liver. The most prominent single gross lesion observed in this study was a pale, yellow-brown, rough, friable liver. There was little variation in color. However, the liver in the very acute cases was more

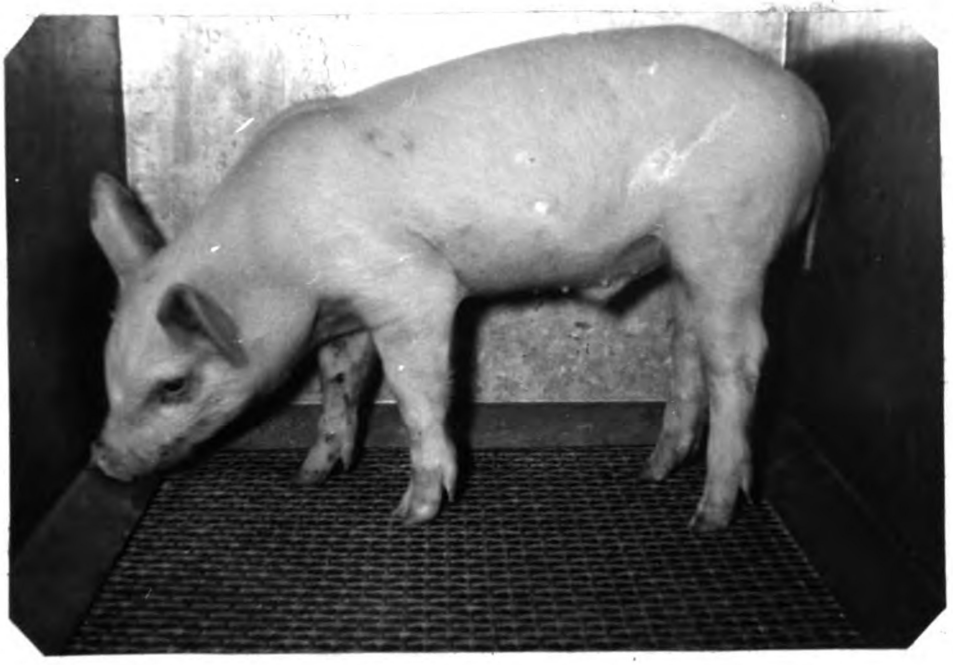

Fig. 1. Comparison of control pig E5930 (above), and pig E5933 from Lot 1 after 12 days (below).

yellow and "fatty" whereas in the less acute cases the texture was more granular and crumbly. Pig E5933 was the exception. This animal was fed a selenium-free ration for 6 days and at the termination of the experiment the gross appearance of the liver was normal.

Microscopically the liver lesions observed can be divided into an acute group where congestion and centrolobular degeneration prevailed, and a more chronically affected group displaying early regenerative activity. All livers except those from the controls manifested fatty changes.

The acutely ill group included seven pigs, all of which died during the first six days. Congestion and acute centrolobular degeneration were especially conspicuous in this group. The congestion was most pronounced in the livers from animals in Lots 4 and 5, although marked individual variation existed. Areas near the capsule were most severely affected. Here the central veins and surrounding sinusoids were packed with erythrocytes. The congestion was not always restricted to the center of the lobule, however. In various sections portal veins were filled with blood and the areas around them contained small amounts of serofibrinous exudate and escaping erythrocytes.

Degenerative changes were more severe in the centrolobular areas. Cloudy swelling was widespread and affected a majority of the central parenchymal cells. More advanced changes were evidenced by numerous pyknotic and karyorrhectic nuclei which were not always restricted to the central portion of the lobule. Increased numbers of Kupffer cells were present in most sections.

Fatty metamorphosis in the liver was widespread and severe. Only in the liver of pig E5934, which succumbed after ingesting 25.9 mg. of

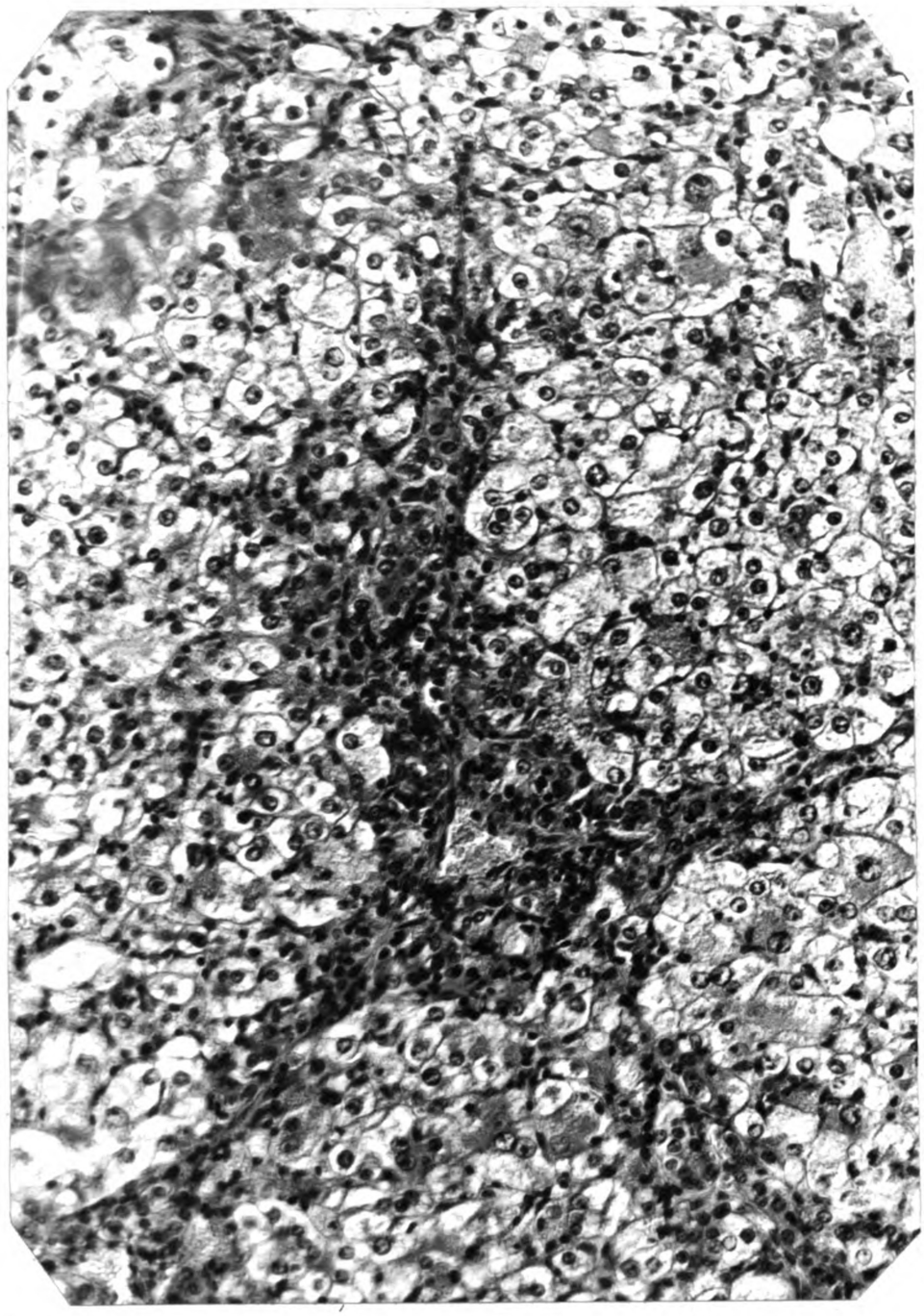

Fig. 2. Attempted bile duct formation at the periphery of a lobule in the liver from pig E5935. H&E stain; X475.

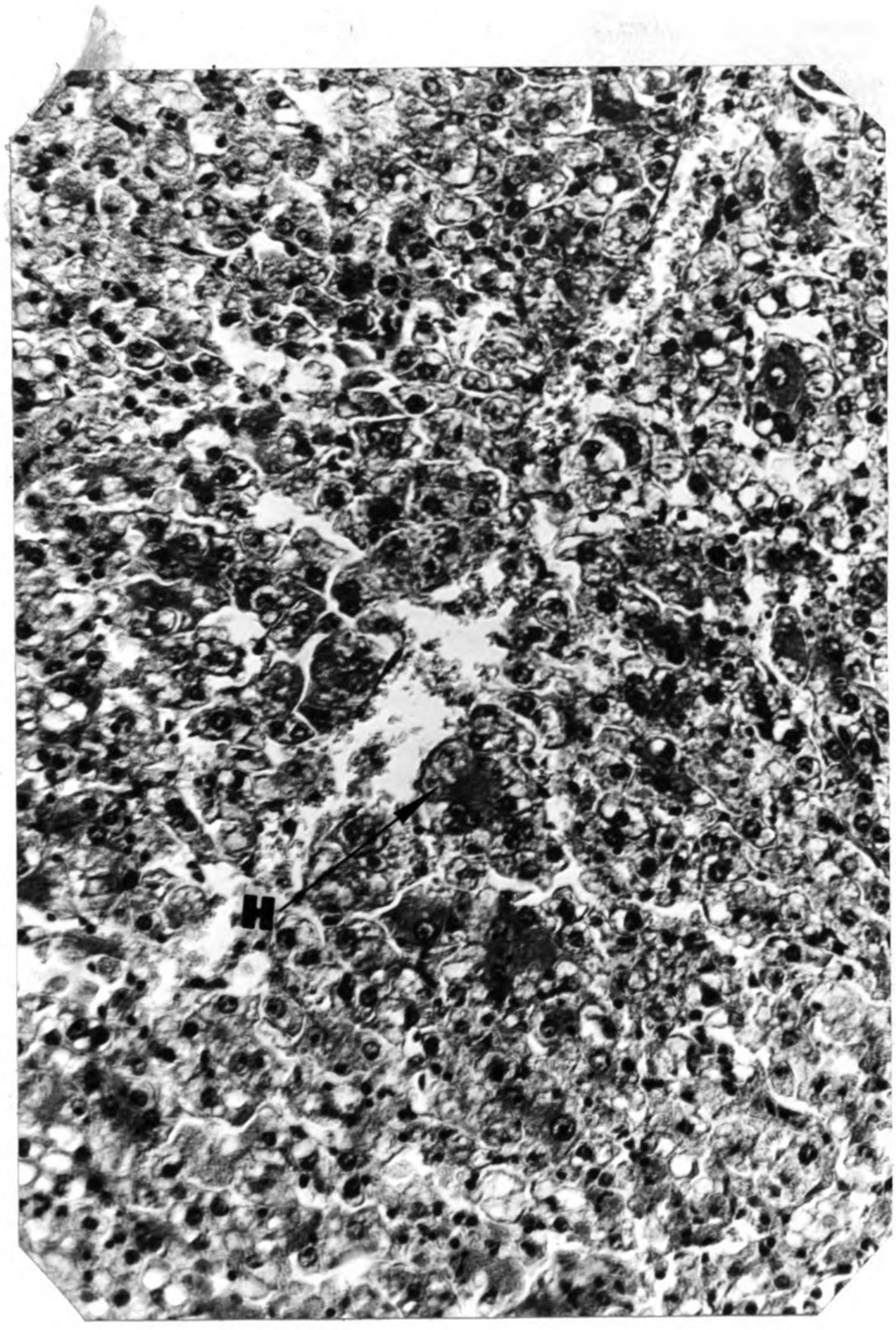

Fig. 3. Centrolobular destruction and large basophilic regenerating hepatic cells (H) in the liver from pig E5935. H&E stain; X570.

selenium, was the fat restricted to scattered individual liver cells. In the rest of the pigs, a few too many fat globules were present in the cytoplasm of almost every parenchymal cell which was not in an advanced stage of degeneration. Therefore, under low-power magnification, a lobule stained with Sudan IV appeared as a red, granular disc encircled by a narrow rim of periportal connective tissue and containing a central vein surrounded by a zone of degenerating cells. Accumulation of fat was generally more pronounced in the peripheral third of the lobule. In addition, the size of the intracellular fat globules were much smaller in the acutely ill animals, whereas in the more chronic cases these globules tended to coalesce until the nucleus was displaced to the border of the cell. However, no "lipodiastaemate" (a large multicellular structure containing a huge central fat droplet) as described by Hartroft (1950) were found.

In the three animals which survived more than 10 days, very early regenerative changes were noted. These included slight increases in collagen and early fibroblast proliferation in periportal areas, apparent attempts at bile duct proliferation (Figure 2), and the appearance of large, often multinuclear, cells (Figure 3) which are associated with regeneration (Schiff, 1956; Beams and King, 1942).

In the gall bladder the most consistent findings were varying degrees of mucous degeneration and desquamation of the mucosal epithelium.

Kidney. Gross lesions in the kidneys were not observed. Ureters and urethra were apparently normal. The urinary bladders were in various stages of contraction and no gross changes were evident.

Histopathological lesions were present in the kidneys of all the selenium-fed pigs and included: congestion, usually in the medulla;

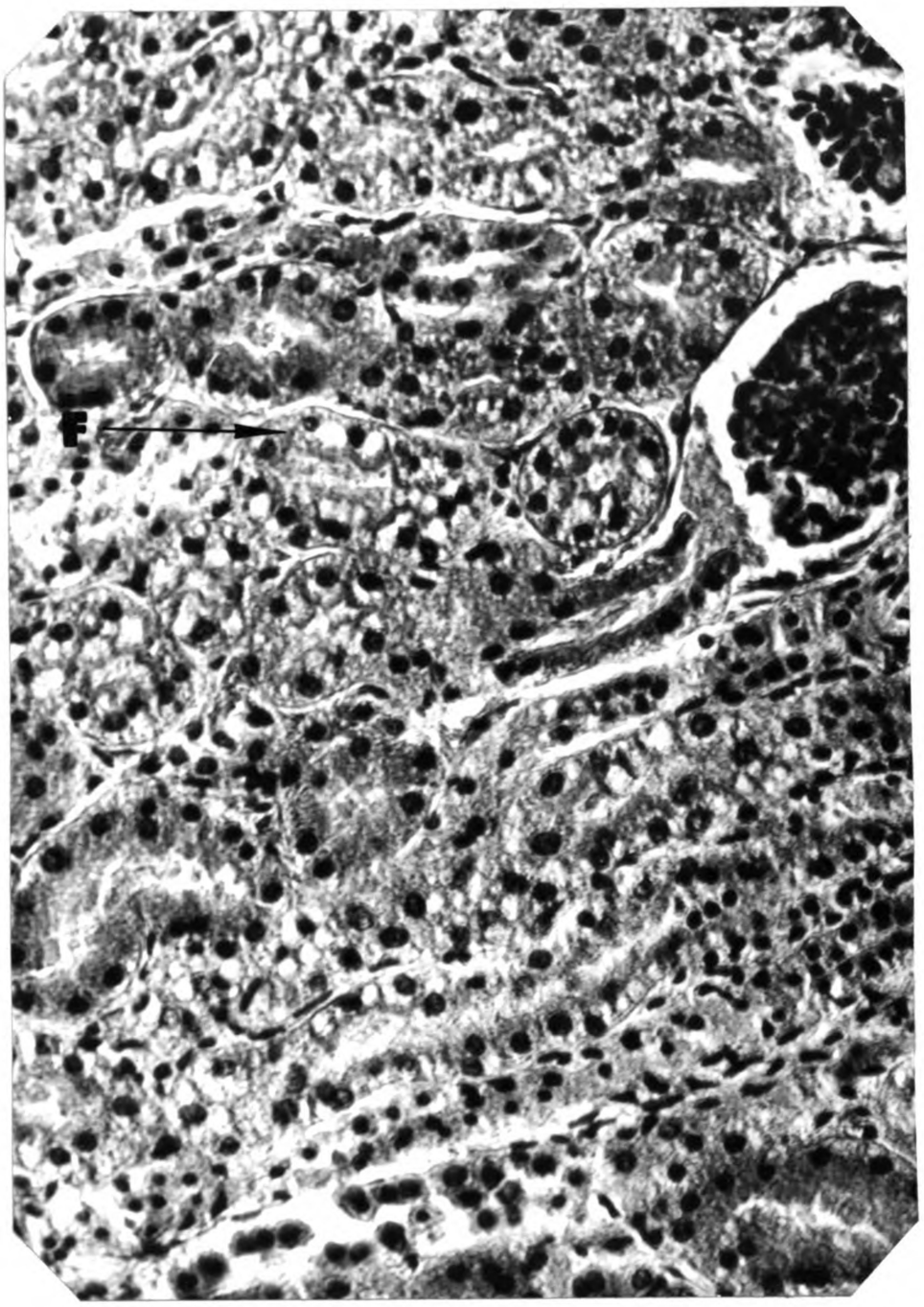

Fig. 4. Renal degeneration (E5936) with fatty metamorphosis of the proximal convoluted tubules (F), and necrosis of the ascending thick segment of Henle's loop. H&E stain; X830.

cloudy swelling and coagulation necrosis of the proximal convoluted tubules; and various degrees of tubular fatty metamorphosis. No distinct relationship could be found between any of the above processes and the amount of selenium fed, although congestive changes were most severe in the acutely ill cases.

Generalized congestion of blood vessels was a predominant feature in the kidneys of the animals that died during the first three days. The degree of congestion was most severe in the tissues from pigs in Lot 5. In the kidneys of these animals the glomerular capillaries were distended and engorged with erythrocytes. The interlobar, and arcuate veins, as well as the capillaries of the medulla, were engorged with erythrocytes. Perivascular edema and occasional interstitial hemorrhages were also noted. In the bladder, moderate congestion of subepithelial capillaries and edema of the muscularis mucosa prevailed.

Degenerative changes varied widely in the kidneys. In the acute cases, the predominant change was cloudy swelling, which was most conspicuous in the loops of Henle. The most severe degenerative changes generally occurred in the ascending thick segments of the loop. In these medullary segments pyknotic nuclei and granular, poorly staining cytoplasm were especially noticeable (Figure 4).

Fatty metamorphosis, which was very pronounced in all but two of the kidneys, affected the convoluted and terminal portions of the proximal tubules almost exclusively (Figure 4). Ascending segments, distal convoluted tubules, and collecting tubules were almost devoid of fat.

The urinary bladder was remarkably free of lesions. Only mild degrees of congestion, edema, and occasional areas of desquamation of transitional epithelium were noted.

Gastrointestinal Tract. At necropsy, the stomachs of the acutely ill animals contained varied quantities of mucus and undigested feed. The mucosa appeared slightly hyperemic and in the fundus small circumscribed brown clots of blood were noted. These areas were present in over half of the animals.

The intestines appeared distended with gas, dark red, and highly congested in the acutely affected animals but more nearly normal in the chronically affected cases. The lumina were usually empty and only in the terminal portions of the colon was fecal matter found. The mesenteric vessels were engorged and dark blue in color. Mesenteric lymph nodes were not unduly enlarged and the esophagus and pancreas appeared grossly normal. No ascitic fluid was noted.

Microscopically the stomach and small intestines exhibited the more marked lesions. Congestion, hemorrhage, leukocyte infiltrations and erosion or desquamation of the epithelial lining were most often found.

Lesions in the stomach were quite similar in all cases. The most severe manifestations generally occurred in the animals fed the largest amounts of selenium, while the pig which had been on a selenium-free diet for 6 days exhibited no obvious lesions.

Congestion in the stomach appeared most severe in the lamina propria. Capillaries were engorged with erythrocytes and often so distended that the whole area appeared hemorrhagic. The mucosal layer was not the only one affected, however. The larger vessels in the submucosa and even in the mesenteries were not infrequently plugged with cells, and thrombi were seen in various vessels. In these areas of severe venous stasis, edema often caused some mild separation of connective tissue fibers. This was most prevalent in the submucosa.

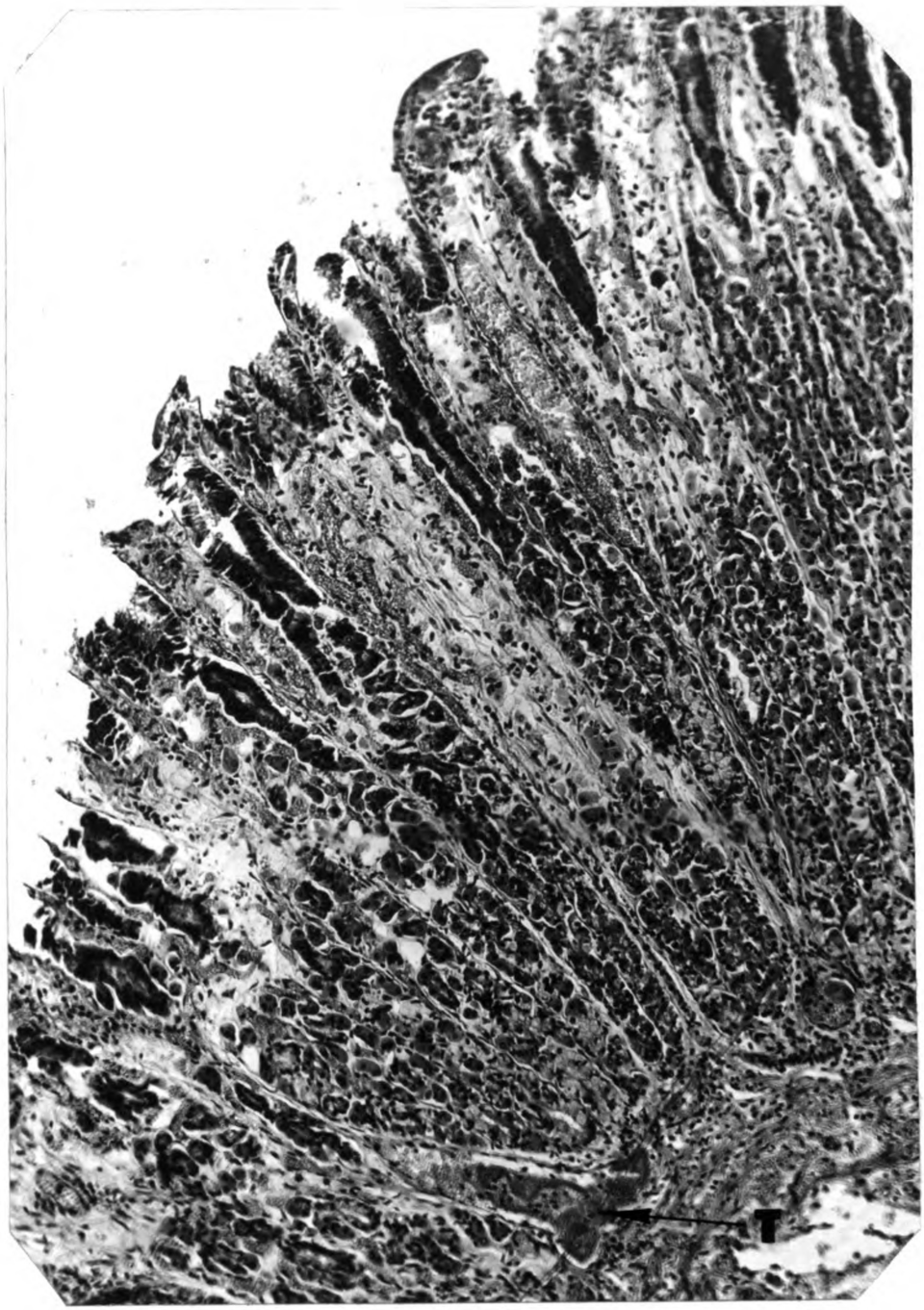

Fig. 5. Degeneration of gastric glands in pig E5936. Note congestion of vessels and thrombus (T). H&E stain; X128.

Degeneration of the gastric epithelium was common in most of the pigs. The degree and extent of damage varied from isolated areas of mild surface erosions to complete denudation and mucous degeneration of large numbers of gastric glands, leaving only the connective tissue skeleton of the lamina propria (Figure 5). Hemorrhage and coagulation or caseation necrosis were usually evident in these areas and the coagulated blood pigments near the surface of the lesion were a vivid yellow-brown. Generally, however, the lesions were not so severe and consisted mostly of mucous degeneration of the surface cells and neck cells, with some sloughing of the former. Distention of the capillaries and mild infiltration of lymphocytes and plasma cells in the lamina propria completed the usual picture.

The lesions found in the small intestine were usually very similar to those found in the stomach. Congestion of blood vessels was pronounced in almost all cases and leukocyte infiltrations of the lamina propria were much more conspicuous than in the stomach. Peyer's patches were congested and hemorrhagic in the acutely affected animals (Figure 6) and areas of hemorrhage into the lumen of the intestine were repeatedly observed.

Denuding of the epithelium of the villi was another common finding in acute toxicosis (Figure 6). In the more chronically affected animals, however, the damage was usually restricted to mucous degeneration and scattered coagulation necrosis with isolated erosions.

Lesions in the colon were relatively mild. They consisted of congestion and a small amount of epithelial erosion. Only pig E5941 demonstrated marked degeneration.

Heart and Lungs. Grossly the heart and lungs exhibited few lesions. No abnormalities of the lungs were observed, while the heart lesions were

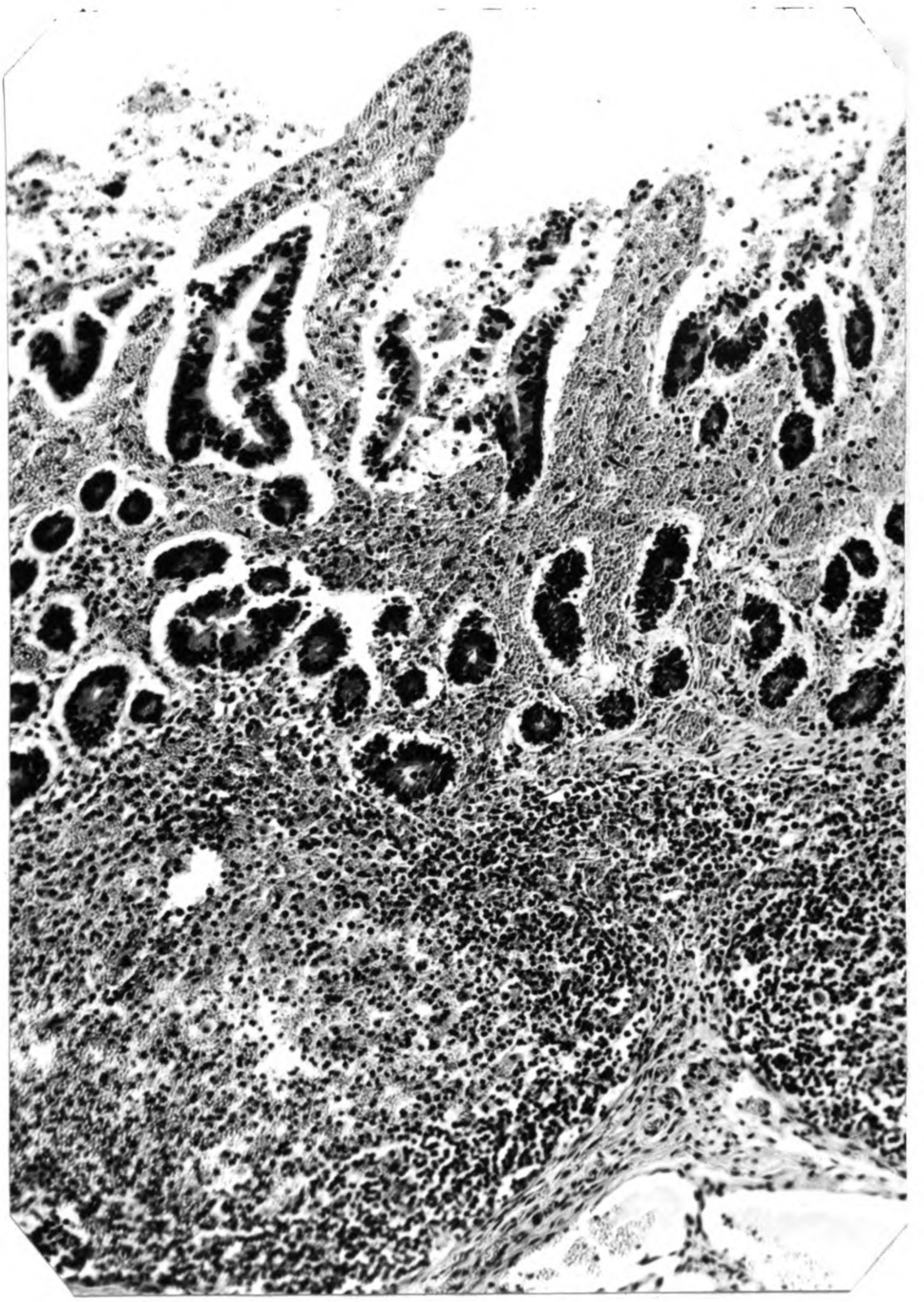

Fig. 6. Denuding of the epithelium from the ileum of pig E5941. Note the hemorrhage and congestion in the lamina propria and Peyer's patch. H&E stain; X150.

usually restricted to epicardial petechial hemorrhages present in the region of the coronary groove. Pig E5932 was the only animal demonstrating any abnormal amount of pericardial fluid. About 5 cc. of clear fluid was seen in this case. Endocardial hemorrhages were absent. Fluid was never observed in the thoracic cavity in demonstrable amounts at autopsy.

Histopathological changes in the heart were restricted to capillary congestion, a few myocardial and epicardial hemorrhages and a moderate amount of fatty metamorphosis. The hemorrhages were usually localized in the perivascular fat of the coronary vessels and were probably due to diapedesis. Evidence of fatty metamorphosis could not be detected in the sections stained with hematoxylin and eosin, but the sections stained with Sudan IV revealed numerous small to medium fat spherules dispersed throughout the myocardial muscle fibers. The spherules often appeared in small clusters around the nuclei of muscle fibers but otherwise were apparently evenly distributed throughout the myocardium. All pigs except the control animals were affected. More advanced degenerative changes could not be demonstrated.

Various degrees of capillary congestion were the only persistent changes found in the lungs. Mild interstitial thickening was noted in pig E5935.

Miscellaneous Tissue Changes. Gross lesions other than those which have already been described were not evident. No enlargement or significant abnormality of the lymph nodes or splenic tissue was noted.

Histopathological changes were noted in the adrenal gland and lymph nodes. In the adrenal gland the medulla usually congested with erythrocytes. Occasionally the zona glomerulosa and zona reticulata were similarly affected. The lymph nodes generally demonstrated mild amounts of subcapsular congestion, hemorrhage, and edema. This was more

pronounced in the acutely affected animals. No clearly significant microscopic lesions were noted in sections of semimembranosus muscle, cross sections of tail and hoof, brain, pituitary gland, thyroid, spleen, ovary, uterus, testes and bone.

DISCUSSION

The results of this study confirmed the findings of Miller and Williams (1940a) in that the single oral lethal dose of selenium (in the form of sodium selenite) in swine is approximately 8 mg. per pound of body weight. At this level death resulted in approximately 48 hours. Pigs E5934 and E5937 demonstrated the individual variability that existed. The former ingested a total of only 25.9 mg. of selenite, yet died after 60 hours, while the latter consumed 93.8 mg. and lived for 120 hours. Furthermore, the report of Fitzhugh et al. (1944) that females are more susceptible to selenium toxicity than males could not be verified in the small number of pigs in this experiment. On the contrary, the two animals which survived for the longest period of time were both females.

Signs of anorexia, vomition, diarrhea, hypothermia, dyspnea, and incoordination were noted, and paralleled the observations reported by Beath (1934) and Miller and Williams (1940a). However, no corneal cloudiness, circling, or "pushing" was observed. All the signs seen, except the chorea, were directly proportional to the amount of selenium administered. Garlicky breath which was noted by several early workers was not detected in these pigs.

Laboratory findings indicated that severe hemoconcentration occurred in the very acutely affected animals, while pronounced cellular necrosis was somewhat more delayed. Hemoglobin values from pigs E5938 and E5940, taken shortly before death, had increased by 43%. Anderson and Moxon (1942) reported similar results in dogs.

The most sensitive index of cellular damage, however, appeared to be the SGO-T levels. The SGO-T determination is used to measure an

enzyme present in tissues normally, but which has been released to the blood stream as a result of breakdown of the cell barrier by necrosis. Cornelius et al. (1959) reported the normal for pigs to be 22.7 ± 5.4 S-F units. This corresponds well with the range of 21 to 49 S-F units in the 12 pigs before the start of this experiment. It was further reported that this test was of value in the diagnosis of white muscle disease (WMD) in lambs and calves. Blincoe and Dye (1959) noted values of 295-2360 S-F units in affected animals while normal calves and lambs ranged between 19 and 99 S-F units. Lagace (1961) obtained somewhat higher results. It was of interest, therefore, to find that, in the more chronically poisoned animals of this experiment, a range of 335 to 870 S-F units was obtained after 9 days on a selenium diet. Two determinations taken from pigs in Lots 4 and 5 showed only a moderate rise (90 and 170 S-F units).

It is not known exactly which tissues caused the marked elevation of SGO-T values. According to Cornelius (1959) only six organs contain significant amounts of glutamic-oxalacetic transaminase in the pig. They are, in descending order: heart, skeletal muscle, kidney, liver, pancreas, and spleen. Of these, the heart, muscle, and kidneys contain more than twice the amount of enzyme than the rest of the organs. Yet, this is not where the most severe histopathological lesions were found. It may be that alterations in cellular permeability occur without the obvious histological signs of cell necrosis.

Other laboratory tests, such as total and differential leukocyte counts, were not significantly altered. SGP-T values were never elevated (in fact they appeared depressed) due probably to the fact that the pig contains significant amounts of this enzyme only in the kidney.

The single van den Bergh test for total bilirubin, which was run on the blood from pig E5932, was within the normal range and thus in harmony with the report of Smith et al. (1940) that no bilirubinemia was ever found in chronically poisoned rats and cats.

Gross lesions observed at necropsy generally paralleled those seen by Draize and Beath (1935) in sheep and cattle affected by "blind staggers" and will not be enumerated in detail. However, there are some differences which warrant further discussion. One is the large degree of fat present in the livers of the pigs fed selenium in this experiment. No mention of fatty livers was made by Draize, who merely described this organ as acutely congested and containing areas of focal necrosis. Rosenfeld and Beath (1946a), on the other hand, made note of the fact that the livers from sheep which died of acute range intoxication and blind staggers were usually soft, yellow, and contained extensive fatty changes and areas of necrosis.

Further disparities in the gross lesions were restricted chiefly to the degree of the hemorrhagic manifestation. None of the pigs in this experiment exhibited the petechial or ecchymotic hemorrhages in the lungs, endocardium, and peritoneum that were reported by Rosenfeld and Beath (1946a) for sheep. Changes in the colon were also not as severe and could be labeled as congestive, rather than hemorrhagic enteritis.

Histopathological changes were most severe in the liver, kidneys, and gastrointestinal tract. In general the lesions found were quite similar to those reported by Miller and Williams (1940a) in one pig killed by 10.3 mg. sodium selenite per pound body weight. However, the heavy deposits of bile and the marked degenerative muscle changes in the heart, as well as the catarrhal pneumonia in the lungs, were not observed.

A summary of microscopic liver lesions included congestive changes, various degrees of centrolobular degeneration, and severe fatty metamorphosis. Of these, the fatty changes probably represented the earliest manifestations of liver damage. Hartroft (1950) demonstrated that these changes take place within 24 hours in rats on a choline-deficient ration. It was therefore not surprising to find marked fatty alterations in the pigs which died after only 48 hours.

At first the fat is apparently present as small globules which have a tendency to coalesce within the cell as time goes on, until finally the spherule becomes so large as to displace the nucleus to the periphery of the cell. This phenomenon was quite marked in the liver sections from this experiment, and the more chronically intoxicated animals displayed much larger globules on the average than the more acutely ill pigs. However, this process apparently was not of sufficient duration to produce the "lipodiastaemata" described by Hartroft in the rat in which several of these distended cells rupture with the formation of an enormous multicellular structure. It was also observed that the fat-containing cells were more concentrated at the peripheral half of the lobules than in the central half, whereas in choline-deficient rats the affected cells appeared more in the midzonal and central areas. Furthermore, almost complete reversal of fatty metamorphosis was achieved in rats by discontinuation of the choline-deficient diet for 3 to 4 days. Yet, no microscopic evidence of diminished fat accumulation was noted in pig E5933 after 6 days on a normal, selenium-free diet, although the gross appearance of the liver was essentially normal. Apparently the hepatocellular damage produced by selenium is of a more severe nature than that produced by a choline-deficient diet and is not easily reversed.

Renal lesions included engorged medullary capillaries, fatty metamorphosis, and various degrees of tubular degeneration. Almost identical observations were made by Miller and Williams (1940a) and Rosenfeld and Beath (1946a). Draize and Beath (1939), however, also reported a mild glomerulonephritis as well as severe degeneration of the collecting tubules of the medulla in cattle and sheep.

Gastroenteric lesions were most severe in the very acutely affected animals. Hemorrhagic ulcerations in the stomach and a severe catarrhal enteritis predominated. The severity of the inflammatory and degenerative changes progressively decreased further down the enteric tract. The colon was only mildly affected.

The results from pig E5933, which was placed on a selenium-free diet for 6 days after ingesting a total of 52.7 mg. of selenium over a period of 12 days, were of particular interest. Although still somewhat emaciated at the time of necropsy, no other gross alterations were observed. Microscopic changes were comparatively minimal. The predominant lesions consisted of fatty metamorphosis in the liver and kidneys, although evidence of necrosis still persisted. Whether this animal would ever have completely recovered is questionable, in spite of the early weight gains. Franke (1935) claimed that the damage to organs of rats fed seleniferous grain for as little as 10 days was never repaired in spite of resumed normal growth. It is also possible that the small amount of arsanilic acid in the basal ration may have had some beneficial effect.

Other toxic substances may produce symptoms and tissue changes somewhat resembling selenium toxicosis. The necrosis of extremities and signs of lameness seen in ergot and fescue poisoning are quite

similar to chronic selenium intoxication. In addition, chemicals such as arsenic, phosphorus, as well as the ingestion of toxic amounts of gossypol, Senecio, Amsinckia (tarweed), and cocklebur may induce signs and tissue changes resembling those of selenium.

In summary the symptoms and gross lesions observed in the pigs of this experiment were generally similar to those reported by previous authors in cattle, sheep, and pigs. However, in this work information on the histological changes were described in more detail than was previously available.

SUMMARY AND CONCLUSIONS

Sodium selenite was fed to 3-week-old pigs to determine the symptoms, hematology and gross and microscopic lesions. One lot was fed a basal ration while in 5 additional lots the basal ration was supplemented with 0.5, 1.0, 2.0, 4.0, and 8.0 mg. selenium per lb. body weight, respectively. The two higher levels were lethal in approximately 48 hours, while the lower dosages were progressively less toxic.

Signs of anorexia, vomiting, diarrhea, hypothermia, dyspnea, and incoordination were noted. Hemoconcentration was present in the acutely affected animals while serum glutamic oxalacetic transaminase values were increased approximately 10 to 20 fold.

The most severe lesions were noted in the groups fed the largest amounts of selenium. Gross tissue changes included: fatty livers; congested blood vessels; congestive catarrhal gastroenteritis, often with gastric ecchymoses and ulcers; occasional epicardial hemorrhages; and cyanosis.

Histopathological manifestations were most severe in the liver, kidneys, and gastrointestinal tract. Fatty metamorphosis, centrolobular necrosis, and congestion were noted in the liver. Degeneration and fatty changes of the renal tubules [illegible] varying degrees of congestion were present in the kidneys. The most evident gastrointestinal lesions included ulceration, exfoliation, and erosion of mucosal epithelium and congestion, hemorrhages and leukocytic infiltration.

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