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GRADUATE COLLEGE

A STUDY OF THE TRANSPORT OF ZINC TO SURGICALLY INFLICTED WOUND SITES

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

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A STUDY OF THE TRANSPORT OF ZINC TO SURGICALLY

INFLICTED WOUND SITES

APPROVED BY

DISSERTATION COMMITTEE

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A STUDY OF THE TRANSPORT OF ZINC TO SURGICALLY INFLICTED WOUND SITES

CHAPTER I

INTRODUCTION

The study of metallic ions and their effect on the living organism has been continuous throughout the recorded history of man. In his primitive form, man recognized the need for salt in his diet and as civilization progressed sodium chloride became an agent of barter. Other significant elements were observed by the more studious. It was noted that lead, mercury and arsenic could cause fatal intoxication.

In the latter part of the nineteenth century the effect of potassium was demonstrated; and by the turn of the century the essential role of calcium, magnesium, sodium, and potassium in body metabolism was an accepted fact. It was demonstrated that a potassium deficiency caused necrotic changes in myocardial fibers. These changes were followed by scar tissue replacement.

At the present time, sodium appears to be one of the metals significant in the public health field. This element, in varying concentrations, is found in most of the potable water supplies in the world (1). The American Heart Association (2) has recommended that

persons on a moderately restricted salt diet use water containing not more than 20 milligrams per liter of sodium salt in their daily water intake. Dahl (3) in a Brookhaven National Laboratory lecture indicated the role of sodium in the hypertensive state. He noted that rats fed on a high sodium diet rapidly attained a hypertensive state marked by nervousness and elevated blood pressure. When these rats were returned to a normal sodium diet, the conditions were relieved. Dahl (3) also mentioned that people who are hypertensive retain more sodium salts on a lean body weight basis than people who are not in a hypertensive state.

The limitation of intake of sodium is an essential part of the physician's treatment in a number of medical conditions such as congestive heart failure, toxemia of pregnancy, cirrhosis with ascites, chronic renal failure with edema, hypertension and others. Schilling et al. (4) have shown that there is an increase in sodium and a decrease in potassium in the fluids present in wounds during healing.

In addition to sodium, several other metals have been subjected to physiological and statistical investigation. Schroeder (5) reported a significant negative correlation between water hardness and cardiovascular mortality rates. Sauer (6) pointed out that in nine of the ten metropolitan and non-metropolitan areas of the United States having the highest cardiovascular mortality rates, no magnesium was found in the water supply. Significantly, in the ten areas of the United States having the lowest cardiovascular mortality rates, magnesium was found in the potable water supplies. It is easily demonstrated that in dogs with complete parathyroid removal, high calcium water may prevent the

animal's developing of the classical state of tetany associated with this condition.

Lewis (7) reported that vanadium dust exposure brought about a lowering of the serum cholesterol level in exposed workers while a control group of non-exposed workers maintained a constant serum cholesterol level. Strain (8) has drawn attention to the presence of vanadium in hard waters and the absence of it in soft waters in certain areas of the United States. Thompson et al. (9) in their spectrographic analysis of air-dried sewage sludge found, on a comparative basis, that the waste water sludges from certain communities in the state of Oklahoma exhibited a high vanadium content. Upon further investigation, they determined that those communities having a high vanadium content in their sludge also had trace amounts of vanadium in their municipal water supplies. This work was substantiated by personnel in the United States Public Health Service, who expressed the opinion that vanadium in the water supply would enhance the lowering of the incidence of dental caries in the community and would also bring about a lowering of cardiovascular mortality rates (10).

The effect of cobalt on both plant and animal life has received much attention. A lcak of this metal in the diet of ruminants produces a deficiency syndrome marked by anemia while an excess of cobalt in experimental animals produces a true polycythemia. Vitamin B-12, which is a hemopoietic factor contains cobalt.

The role of inorganic ions in enzyme-catalyzed reactions had, for some years, been of special significance to the biochemist. Most workers feel that, like vitamins, the trace elements or micronutrients

perform their most important functions in the nutrition of plants and animals by assuming an activation action in the enzyme system. The variety of hexokinases which have been studied in detail require magnesium ions as the activity metal of choice. Manganese will substitute for magnesium in many cases but the incidence of enzymatic activity is somewhat lowered (10).

In many cases, it has been found that some enzymes may require double ions; that is, when magnesium occupies one site of the enzyme along with adenosine triphosphate (ATP) and a potassium ion occupies the other site of the enzyme, the most active form of hexokinase results. This appears to be the case in fructokinase. The affinity of the enzyme for the magnesium ATP complex is five times greater in the presence of potassium ions. The presence of sodium ions, instead of potassium ions, will cause inhibitory effects.

Warburg and Christian (11) found that inhibited yeast enzyme could be reactivated by the addition of zinc, cobalt, ferrous or cupric ions. Iron has been shown to be essential to other enzyme systems including the aldolase of Claustridium perfringens. Some of the metals which have been shown to have an active role in enzymatic activity are magnesium, calcium, cobalt, nickel, iron, and manganese. Zinc and copper have also been shown to be enzyme activators. Copper activates tyrosinase, lactase and ascorbic acid oxidase, while zinc appears to be primarily responsible for the activity of carbonic anhydrase, alcohol dehydrogenase, dehydropeptidase, tripeptidase, and carosinase, among others (10).

Schroeder (12,13) investigated the possible role of many "non-essential" trace elements and reported on the utilization of these metals and their toxicity. In a series of spectrographic investigations, Tipton and Cook (14,15) examined the distribution of trace metals in various organs of the body. Sunderman (15) studied the carcinogenesis of nickel in rats, and Gilman et al. (17,18) investigated the carcinogenicity of cobalt, copper, iron, and nickel compounds in man. Butt et al. (19) studied trace metals in human serum and blood and identified copper, zinc, iron, calcium, barium, aluminum, and silver as regularly occurring elements. D'Alonzo and Pell (20) studied the variation of metallic components in the blood of myocardial infarction patients and found nickel to be of significance. In their investigation, 19 of 20 patients who had experienced a myocardial infarction exhibited a higher nickel content in their blood serum than did a group of controls who had experienced no myocardial infarction.

Of the various trace metals mentioned thus far, zinc has probably received more consideration than most of the others. It has been found to be present and essential to all living tissues. The whole body of a 70 kilogram man contains approximately 2.2 grams of zinc with the value ranging from 1.4 to 3.0 grams (9). This corresponds to an average body concentration of 20 to 30 microgram per 100 milligram of body weight. The concentration of zinc in the fetus, in which one fourth of the zinc is found in the liver and spleen, changes very little during fetal life. If a newborn animal is fed on later milk instead

of on colostrum the total body zinc value declines. This is probably due to the fact that colostrum contains four or five times as much zinc as later milk does (21).

It has been reported that there are 859 micrograms of zinc per milligram of wet tissue (plus or minus 96 microgram) in the normal human prostate gland and that this value is significantly less in a prostate gland affected by carcinoma (22). Zinc values have also been reported to be reduced in diabetes (dithizone), in acute and chronic infections such as pneumonia, in malignancy, lymphomatous disease and in maximum ambient temperature elevations (10).

There is a significant depression in serum zinc, with a high urinary excretion, in humans having Laennec's cirrhosis. There is increased serum zinc reported in individuals with hyperthyroidism, hypertension, polycythemia vera, sickle cell anemia and eosinophilia, as well as after the administration of adrenalin or thyroxine or after irradiation with x-ray (10).

Prasad et al. reported that there is a zinc deficiency in Egyptian dwarf males having hypogonadism (23). Vallee stated that all other findings, all except the testicular atrophy could be reversed by the addition of zinc to the diet (10).

Vallee reported that there are 120 parts per million (Micrograms per 100 milliliter) of zinc in human blood plasma. The leucocytes contain 25 times as much as the erythrocytes. The erythrocytes of the newborn contain only about 25 per cent as much as the erythrocytes of adults. Patients with anemia show a marked increase in the leucocyte

zinc values; in patients with acute or chronic lymphatic or myelogenous leukemia, there is a decrease to as little as 10 per cent of normal value. Zinc is also found in blood serum and in the contents of the eyeball (10).

Zinc has been shown to have a growth stimulating effect on pullets and to be necessary to the growth and well being of the laboratory rat (24). It has also been shown the zinc prevents and cures parakeratosis in pigs (25).

In fresh tissue of the rat, zinc has been found to be present in the following amounts expressed as parts per million: brain, 18; heart, 21; kidney, 23; liver, 30; lung, 22; muscle, 12; pancreas, 33; spleen, 24; testes, 22; prostate, 223. When zinc in the rat tissue is found to be less than 0.5 microgram per gram, degenerative changes can be expected (10).

In mice and dogs given zinc-65, the most rapid uptake of this material during the first 160 hours occurred in the liver, pancreas, kidney, muscle and skin. The isotope could be detected for as long as eight months after the administration. In the pregnant female dog, more than half of the administered dosage passed freely through the placenta and was taken up by the fetus while the remainder was transferred to the offspring by way of colostrum (9).

Mawson et al. (22) showed that zinc-65 is firmly bound to the tissue and cannot be removed by dialysis or by the administration of ethylenediametetraacetic acid (EDTA). Zinc in serum is completely undialyzable. It is attached partly to the globulin in a firmly bound form and to the albumin in a loose complex. The zinc-albumin complex,

according to Vikladh is primarily concerned with zinc transport (26).

The activity of carbonic anhydrase in tissue increases simultaneously with that of the zinc content. It is known that zinc activates carbonic anhydrase and that the lack of zinc leads to the cessation of respiration (9). Since in wound healing an increased metabolic rate would appear to be helpful, an increase in carbonic anhydrase, and consequently an increase in zinc, could be predicted. However, a satisfactory correlation between enzyme activity and zinc content cannot be made at this time. Neither alkaline nor acid phosphatase activity show any relationship to total body zinc content (10).

It was noted by Strain et al. (8) that in rats on a zinc supplemented diet both wounds and burns healed faster than in control rats. In additional studies using zinc-65 as a radiotracer, it was noted that there is an accumulation of the radioisotope at the edges of the healing wound.

A rapid and marked decrease in the hair zinc levels has been observed in cases of burns or wounds (25). Return to the normal values was a relatively slow process requiring a period of several months.

It has been suggested that zinc exerts its most important function biologically by activating the enzymes concerned with protein synthesis and carbohydrate metabolism and, acting in such a manner, appears to be essential to tissue growth and tissue repair.

Pories et al. (27) demonstrated that the oral administration of zinc sulfate decreased the time required for wound healing following the excision of recurrent pilonidal cysts and suggested that zinc may be

incorporated into the enzyme systems active at the wound site. It was also suggested that zinc may be transported from zinc reservoirs to the area where and when it is needed for tissue growth and repair.

CHAPTER II

PURPOSE AND SCOPE

The literature seems to indicate that appreciable work has been done and much information is now available in specific areas of mineral metabolism. Considerable advances have also been made in our knowledge of traumatic conditions such as those associated with the infliction of wounds and their subsequent healing. However, no previous investigators have conducted or reported comprehensive investigations into the function of zinc in the process of wound healing. The importance of this metal in this capacity was suggested by the work of Grillo et al. (28) which indicated a rapid increase in the enzymatic activity at the wound site. Since zinc is a known enzyme activator, its necessity and presence in areas experiencing high enzymatic activities such as protein synthesis and tissue growth is suggested. Further indications of the possible importance of zinc at the wound site were presented by Pories et al. (27) in their work with zinc-65 and by Strain et al. (27,28,29) in their work on skin repair. Both studies demonstrated a mobilization of zinc into the wound area. In view of these findings, a thorough understanding of the wound healing process requires an evaluation of the zinc activity at the wound site. It was this requirement which provided the

impetus for our research.

More specifically, the purpose of this investigation was to study the alterations in the zinc content of the wound fluid and plasma associated with surgical wounds in both the adult canine and the rodent.

CHAPTER III

METHOD AND PROCEDURE

From March, 1966 to February, 1967, a series of studies was conducted at the Oklahoma Medical Research Foundation under the auspices of the U. S. Army Research and Development Command to determine whether there is a change in zinc levels in wound plasma and wound fluid following the implantation of stainless steel wire mesh cylinders using the methods of Schilling et al. (30) and White et al. (31). The first phase of the investigation involved the use of 14 adult male mongrel dogs (16 to 20 kilogram each) which were randomly divided into three successive studies designed as an initial range finding study followed by intensive examinations of the areas of interest. The second phase of the investigation involved the use of 84 white male rats of the Holtzman strain averaging 250 grams in weight. These rats were randomly divided into groups of 21 each. The second species of animal was used for comparison of results obtained in our dog studies and to permit certain deviations from the initial procedure, if desirable.

In order to minimize the possibility of contamination in our quantitative evaluations of zinc, proper and effective cleaning procedures were institutes and disposable syringes, needles and test tubes were used. Other possible sources of zinc as a contaminant

were eliminated by using Pan Heparin as an anti-coagulant, parafilm instead of rubber stoppers as a closure and triple distilled, deionized water as a diluent. Smoking in the analytical laboratory was prohibited.

For the collection of wound fluid right circular cylinders constructed of stainless steel #46 foundation mesh were used. These measured 5 centimeters in length and 1 centimeter in diameter. For the implantation of these cylinders rigid surgical procedures and techniques were followed.

For implanation of the cylinders, the dogs were weighed and injected intravenously in either front leg with 1 milliliter of Diabutal for each five pounds of body weight. This dosage provided effective surgical anesthesia. Following this, the hair on the back was clipped and the dog was placed on the surgical table with the abdomen down and tied. The dorsal area to be used for the implantation was washed thoroughly with surgical soap, shaved, washed with water to remove the excess soap, finally washed with 70 per cent isopropyl alcohol, and draped with sterile towels, leaving exposed only the area in which the cylinder implantation was made.

Two 1.5 inch midline incisions were made on the dorsal side, one placed anteriorly and the other posteriorly, in order to provide four quadrants in which to implant cylinders. Blunt dissection was employed to create the single furrows beneath the panniculus into which each cylinder was inserted with a trocar. Four or five cylinders were implanted in each quadrant. When the implantations were

completed, subcutaneous and cutaneous closures were made with number 4.0 cotton sutures. The drapes were then removed and the dog was returned to a recovery cage.

Wound plasma (blood plasma at the implantation) from each dog was obtained from a 5 milliliter sample of venous blood, drawn with a disposable syringe and needle and placed immediately into a heparinized tube to prevent clotting. Wound fluid was obtained by aspirating the implanted cylinders, using disposable syringes and needles.

Samples of venous blood and wound fluid were drawn from each of the four dogs in Group 1 once each week to determine possible changes in zinc levels on a weekly basis after surgical insult.

Since the zinc levels in the plasma zinc showed no appreciable change on samples procured on a weekly basis; and since zinc levels in the wound fluid showed a gradual decline, it appeared that our study should be modified so that samples obtained on a daily and hourly basis be evaluated.

The plan followed for each of the six dogs in Group 2 provided for drawing plasma samples each day after implantation. Wound fluid samples were obtained each week. In Group 3, samples of venous blood and wound fluid were drawn from each of the three dogs 1, 2, 3, 6, 12, 23, 29, 35 and 48 hours after cylinder implantation. In one dog, withdrawal was made up to 168 hours after implantation.

For our rat studies, 84 carefully selected white male Holtzman strain, rats averaging 250 grams in weight were divided into two groups of 42 rats each. They were housed in stainless steel cages, the covers

of which were teflon coated to insure against the possible ingestion of zinc from the covers usually provided. Twenty-one of the rats in Group 1 were used as controls. The amount of food ingested and water consumed by each rat each day was determined. The daily excretion of urine and feces was determined. The weight of the feces was on a dry weight basis using a drying temperature of 105 C for 12 hours or to a constant weight.

Zinc levels and quantities of urine and feces excreted and drinking water and food ingested were determined (Table 4, Appendix A) .

For implantation of the cylinders in rats ether was used to produce surgical anesthesia. After the rat was anesthetized, the hair was clipped off the back, the area was washed thoroughly with surgical soap, then shaved and finally rinsed with 70 per cent isopropyl alcohol. Ths incision for implantation was made in the posterior dorsal midline. Blunt dissection was utilized to create a furrow beneath the skin adequate for cylinder implantation. Strict surgical procedures and techniques were used at all times. The stainless steel cylinders, one on each side of the incision, were inserted with a Russian dressing forceps following which closure was made with Michel wound clips. The rat was then placed in its proper cage for recovery.

To obtain samples of wound plasma and wound fluid from the rat our procedure had to be changed from that used for the dog. It was necessary to sacrifice each rat when sampling was done.

In the procedure we used, the rat was anesthetized with ether, the cylinders were aspirated with a 5 milliliter disposable syringe

fitted with a 1.5-inch 20-gauge disposable needle; and, finally each cylinder was removed through an incision made at the base of the cylinder and frozen for further planned biochemical examinations of the contents.

After the cylinders were removed, the rat was placed on his back and a mid-line 1.5 inch long abdominal incision was made. The viscera were moved to one side and the rat was exsanguinated through the abdominal aorta by means of a 10 milliliter disposable syringe fitted with a 1.5 inch 20 gauge disposable needle. The blood was immediately and carefully transferred to a heparinized tube and centrifuged for the procurement of the plasma.

As previously stated, 21 rats of the first group of 42 were used as controls. Each of the remaining 21 rats of this group had 2 cylinders implanted as described.

Three rats of each group of 21 were sacrificed each week - the control rats for the collection and determination of plasma zinc. The 21 experimental rats provided weekly samples of blood plasma and wound fluid.

Each of the second group of 42 rats was used for the implantation of two cylinders. These rats were sacrificed for wound plasma and wound fluid: 1, 3, 6, 9, 12, 18, 24, 48, 72, 96 and 120 hours after implantation.

All wound plasma and wound fluid samples were analyzed for zinc content by the atomic absorption spectrophotometric method. For such determination, the wound fluid and wound plasma collected from each dog and rat were diluted with distilled, deionized water using 5 parts of

water for 1 part of test sample. This diluted sample was then aspirated into a Hetco burned using a hydrogen-air mixture as the fuel and ozidant. The burner was mounted in a 5-pass Jarrell-Ash Atomic Absorption Unit. A zinc hollow cathode tube, manufactured by Westinghouse Electric Company, provided the photon source and was maintained at nine milliamperes throughout the experiment. The voltage on the phototube was held at 900 volts. Figure 1, Appendix B shows a typical zinc curve obtained during the procedure described.

CHAPTER IV

OBSERVATIONS AND DISCUSSION

In a comparison of the zinc levels in wound plasma and wound fluid and any changes detected after the implantation of stainless steel cylinders in dogs, it is interesting to note that there appers to be little if any change in wound plasma zinc but a gradual, significant, and continued decline of the zinc values in wound fluid collected on a weekly basis. This statement is supported by the information given in Table 1 of Appendix A and Figure 2 of Appendix B.

Evidence demonstrates the increased concentration of zinc in a wound area. Since wound healing is both an acute and chronic process and because the inflammatory response begins immediately after injury, it was decided to collect plasma on a daily basis.

There is a distinct elevation of plasma zinc concentration detected 24 hours (1 day) after implantation, reaching a peak within two days and continuing with high values for several days (Table 2 of Appendix A and Figure 3 of Appendix B).

The decline of zinc values for wound fluid is evident from the seventh day after implantation and continues at least through the fourth week as reflected in Table 2 of Appendix A. To determine, more accurately, how soon after injury that changes in the zinc level in plasma

and wound fluid can be detected, we collected samples within hours after the implantation. We detected a distinct elevation of wound plasma zinc within in 12 to 30 hours or earlier following implantation as shown in Table 3 of Appendix A and Figure 4 of Appendix B.

These elevated values appear to return toward a normal level beginning the third day after implantation. Wound fluid zinc values are found to be elevated within one hour after implantation and continue to be elevated for a variable number of days as reflected in Table 3 of Appendix A and Figure 5 of Appendix B.

Comparing the zinc values in plasma and wound fluid in rats after the implantation of cylinders with those observed in dogs treated in an identical manner, the data indicates similar changes in these two species. Zinc concentrations in plasma and wound fluid, when collected on a weekly basis, indicate no noteworthy differences between the profiles obtained on dogs and rats either in the control or wounded groups, Tables 1 and 5, Appendix A and Figures 2 and 6, Appendix B. When collected on an hourly basis after implantation, however, a rise in zinc level in both the plasma and wound fluid was detected as early as the first hour after implantation and continued at least through the sixth hour in rats as shown in Table 6 of Appendix A and Figure 7 of Appendix B.

Such observed changes in both the dog and rat suggest that zinc participates in the early inflammatory process of wound healing. The fact that increased zinc values in wound plasma and wound fluid occur simultaneously and quite early after the surgical production of a wound

further suggests that the organism can utilize zinc from a circulating or fixed stores of zinc at the local level. Vallee (10) indicates a transfer of zinc from the spleen or the liver.

The fact that wound healing is retarded in cases of zinc deficiency suggest that zinc may be essential to healing, but the exact mechanism of its use in healing is still speculative. Some would propose an explanation based on enzyme activity and the place of zinc in enzyme systems. The suggestion by some surgeons that zinc is an accelerator substance and when ingested or applied locally to wounds promotes healing seems untenable. The fact that zinc deficiency decelerates wound healing does not mean that an excess is an accelerative factor. Little is known of the mechanisms of action and interaction of trace metals in the biological systems. The need for more extensive investigation is evident.

Exact and precise analytical procedure in trace metal determinations require caution. Our studies required that we be aware of potential contamination. Zinc is prevalent in reagents, on glassware, rubber stoppers, on our hands, and in the air. It is difficult to develop precise quantative and reproducible procedures regarding microgram amounts of trace metals in small samples. Atomic absorption spectrophotometry offers a valuable tool, if properly used. We believe our study has contributed to the refinement of a method of analysis and the potential application of it to a major disease process, mainly wound trauma.

CHAPTER V

SUMMARY

This investigation was concerned with the evaluation of zinc mobilization in the wound fluid and plasma resulting from or associated with surgically inflicted wounds. Our studies, which involved the implantation, surgically, of stainless steel mesh cylinders as wound fluid collecting devices, employed 14 adult male mongrel dogs and 84 adult male Holtzman albino rats divided into serial studies covering various portions of the wound healing sequence.

Based on the results of the various determinations conducted throughout this investigation, the following observations were drawn:

1. There was a rise in the zinc level of the plasma and wound fluid in the dog within 12 hours after the implantation. This rise continued for several days and was followed by a decline which continued through the fourth week.
2. In the rat there was a plasma and wound fluid zinc level rise which was detected after the first hour following implantation and which continued for 6 hours after which there was a decline which continued through the fourth week.

3. At the end of the fourth week the plasma zinc levels had returned to control values, where they remained.
4. The changes in plasma and wound fluid zinc levels were rapid and transitory in both the dog and the rat.
5. Measurements of the zinc intake of a growing adult Holtzman albino rat in water and feed and of the zinc output in urine and feces demonstrated a positive zinc balance of 52 micrograms of zinc per day.

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APPENDIX A TABLES

TABLE 1

WOUND PLASMA AND WOUND FLUID ZINC LEVELS
FROM THE WEEKLY DOG STUDY

Time in Weeks	Wound Plasma Zinc Conc, ug/100 ml.					Wound Fluid Zinc Conc, ug/100 ml.				
	D191	D192	D193	D194	Avg.	D191	D192	D193	D194	Avg.
0	90	78	82	66	77	-	-	-	-	-
1	90	79	76	72	77	106	89	80	102	94
2	81	77	52	78	72	91	69	58	67	71
3	84	68	74	72	72	94	64	71	79	74
4	96	68	78	72	75	87	54	27	51	55

TABLE 2

WOUND PLASMA AND WOUND FLUID ZINC LEVELS
FROM THE DAILY DOG STUDY

Time in Days	Wound Plasma Zinc Conc, mg/100 ml.							Wound Fluid Zinc Conc, mg/100 ml.						
	D1	D2	D3	D4	D5	D6	Avg.	D1	D2	D3	D4	D5	D6	Avg.
0	87	93	54	72	60	60	71							
1	120	150	54	126	126	96	112							
2	144	120	120	114	210	126	154							
3	108	120	210	132	84	96	125							
4	87	114	132	-	114	-	111							
5	60	110	114	-	-	-	86							
6	60	84	54	70	60	54	64							
7								72	81	99	60	54	51	69
14								56	39	30	30	48	30	54

TABLE 3

WOUND PLASMA AND WOUND FLUID ZINC
LEVELS IN THE HOURLY DOG STUDY

Time in Hours	Plasma Zinc, ug/100 ml. Dog 211	Dog 212	Dog 213	Dog 214	Wound Fluid Zinc, ug/100 ml. Dog 211	Dog 212	Dog 213	Dog 214
0	102	117	102	84				
1	102			102				228
2	102			102	672			
3	102			102	672			240
6	108			84				198
12	108	117	108	96	450	312	150	132
18		150	102		270	180	174	
23	132			120				156
24		96	96			180	138	
29	168				300			
30		102	93			138	117	120
35	150				282			
36				72		126	117	132
42		93	102	72		120	96	102
48	108			66	240	111	111	84
54				66				66
60				114				84
67		111	108			117	93	
72				54				96
86				54				66
90		120				96	78	
96				40				40
168				75		126	66	75

TABLE 4

BASELINE STUDY OF RAT METABOLISM

	Water Intake, ml.		Food Consumed, gms.		Urine Excreted, ml.		Dried Fecal Excretion, gms.	
Day	Rats A and B	Rats C and D	Rats A and B	Rats C and D	Rats A and B	Rats C and D	Rats A and B	Rats C and D
1	65	75	35	35	30	35	3.0	3.0
2	45	55	18	18	30	18	2.5	3.0
3	60	70	30	30	20	30	3.0	4.3
4	60	40	30	30	20	30	3.4	6.8
5	40	70	31	31	20	31	7.2	8.0
6	40	60	35	37	20	37	3.0	3.0
7	50	80	27	28	15	28	3.1	3.0
8	70	75	30	31	30	31	4.0	2.5
9	70	75	32	31	30	30	2.3	4.2
10	60	60	25	35	20	28	4.0	7.0
11	70	65	18	29	15	31	2.7	4.6
12	60	75	23	32	20	27	6.5	7.0
13	75	60	29	31	18	31	3.5	7.5
14	75	80	29	30	17	25	2.7	3.2
Avg./Rat/Day	32		15		13		2.1	

Average daily zinc intake (food and water) is 86.0 micrograms.
Average daily zinc output (urine and fecal) is 34.0 micrograms.

TABLE 5

CONTROL PLASMA, WOUND PLASMA, AND WOUND FLUID
ZINC LEVELS FROM THE WEEKLY RAT STUDY

Time in Weeks	Control Plasma Zinc, ug/100 ml.				Wound Plasma Zinc, ug/100 ml.				Wound Fluid Zinc, ug/100 ml.			
	Replicate:				Replicate:				Replicate:			
	1	2	3	Avg.	1	2	2	Avg.	1	2	3	Avg.
1	123	168	123	138	123	114	-	118	48	90	48	62
2	138	156	108	134	102	120	120	114	60	75	51	62
3	150	138	138	140	156	102	108	128	54	72	77	65
4	170	138	102	137	120	140	128	129	45	72	54	57
5	138	180	126	148	132	132	126	130				
6	144	126	126	132	144	126	126	132				

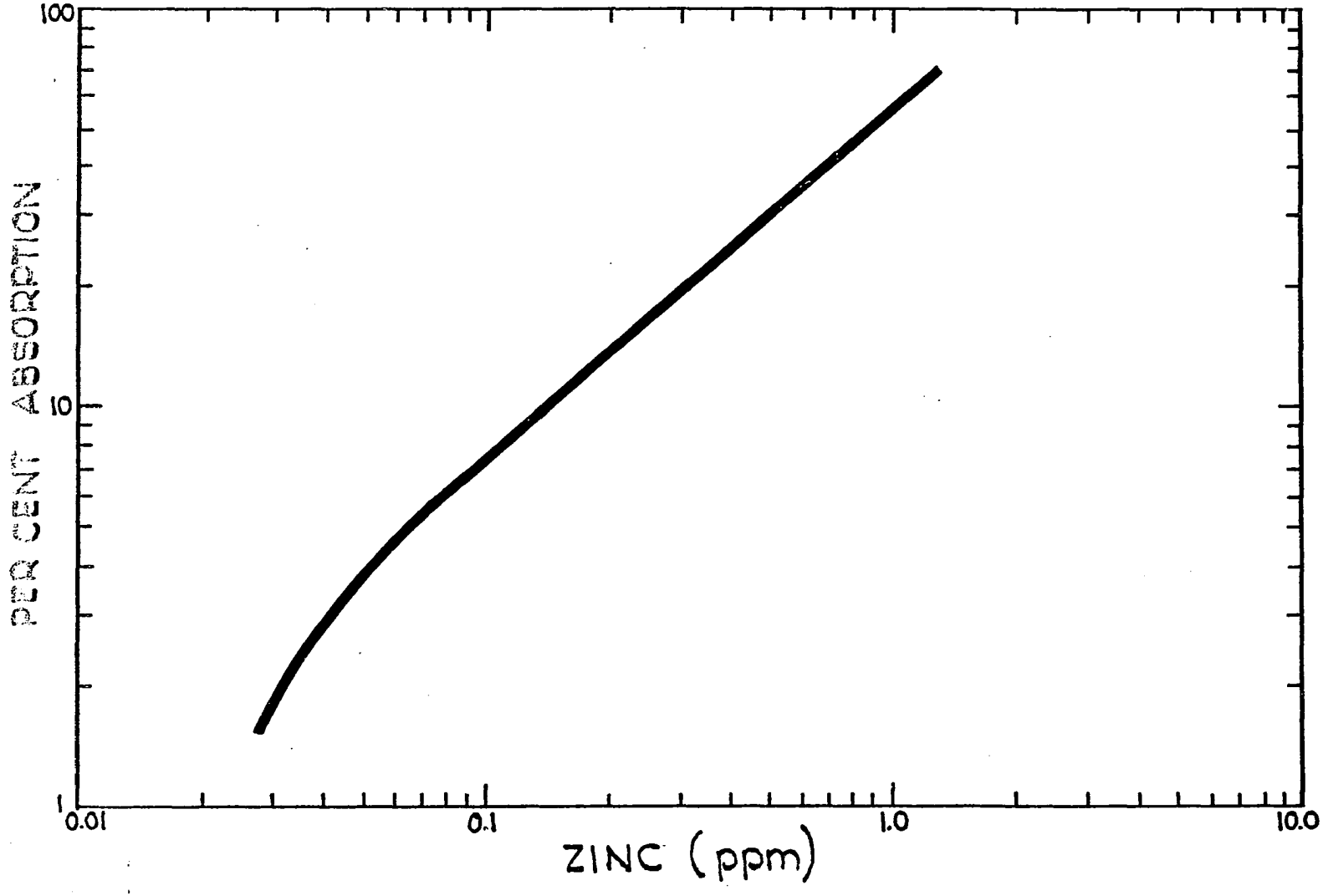
TABLE 6

WOUND PLASMA AND WOUND FLUID ZINC LEVELS
FROM THE HOURLY RAT STUDY

Time in Hours	Wound Plasma Zinc, ug/100 ml. Replicate: 1	2	3	Avg.	Wound Fluid Zinc, ug/100 ml. Composite Sample from Three Rats
0	114	126	-	120	
1	126	138	150	138	150
3	150	126	126	134	150
6	174	150	-	162	162
9	102	102	102	102	124
12	102	102	111	106	120
18	114	102	-	108	138
24	114	102	102	106	114
36	126	126	-	126	126
48	102	-	102	102	114
72	138	126	126	130	114
96	127	127	-	127	112
120	127	127	127	127	112

APPENDIX B FIGURES

Figure 1. Typical curve showing zinc standards by atomic absorption spectrophotometry.



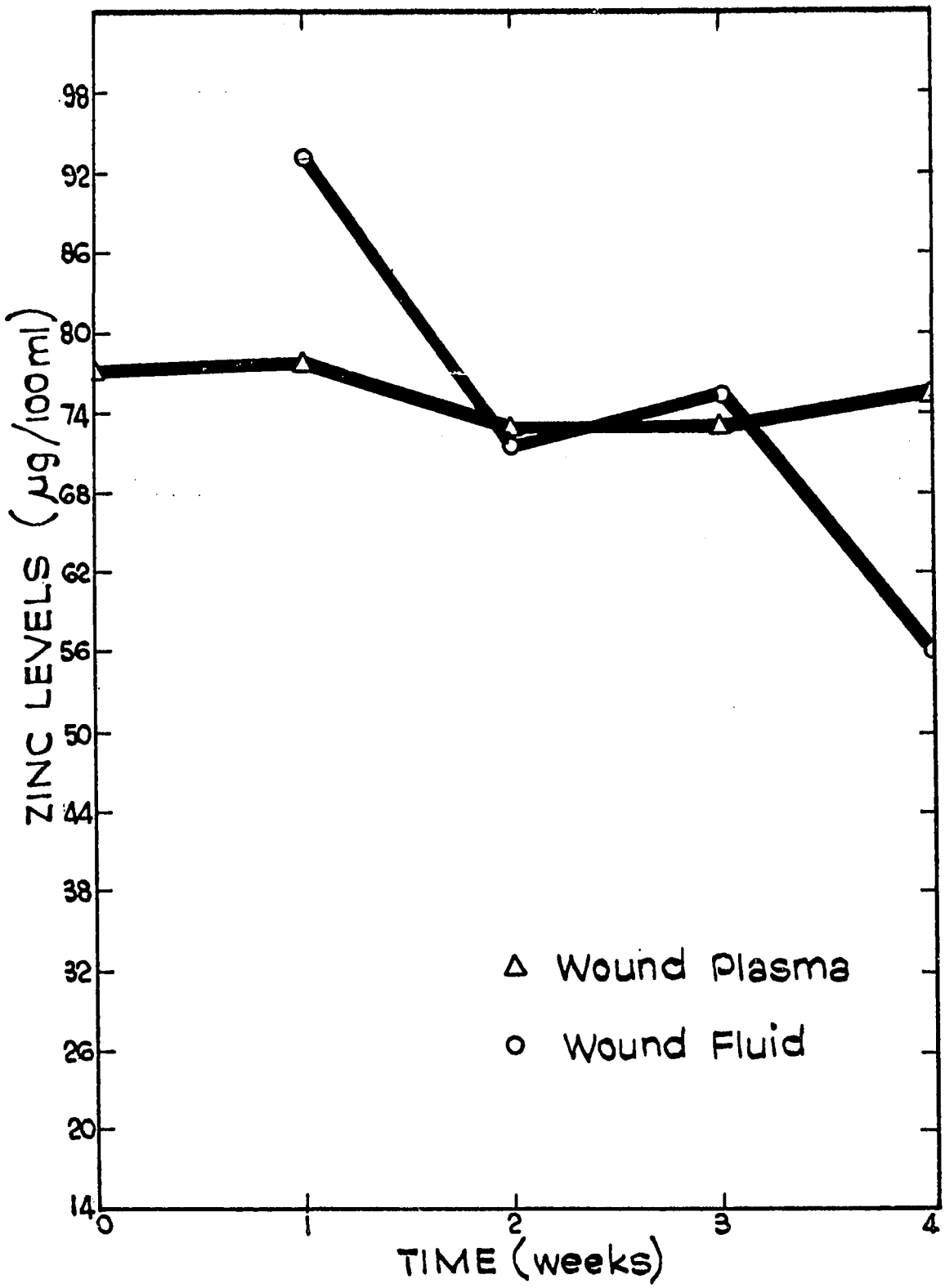


Figure 2. Average wound plasma and wound fluid zinc levels from the weekly dog study.

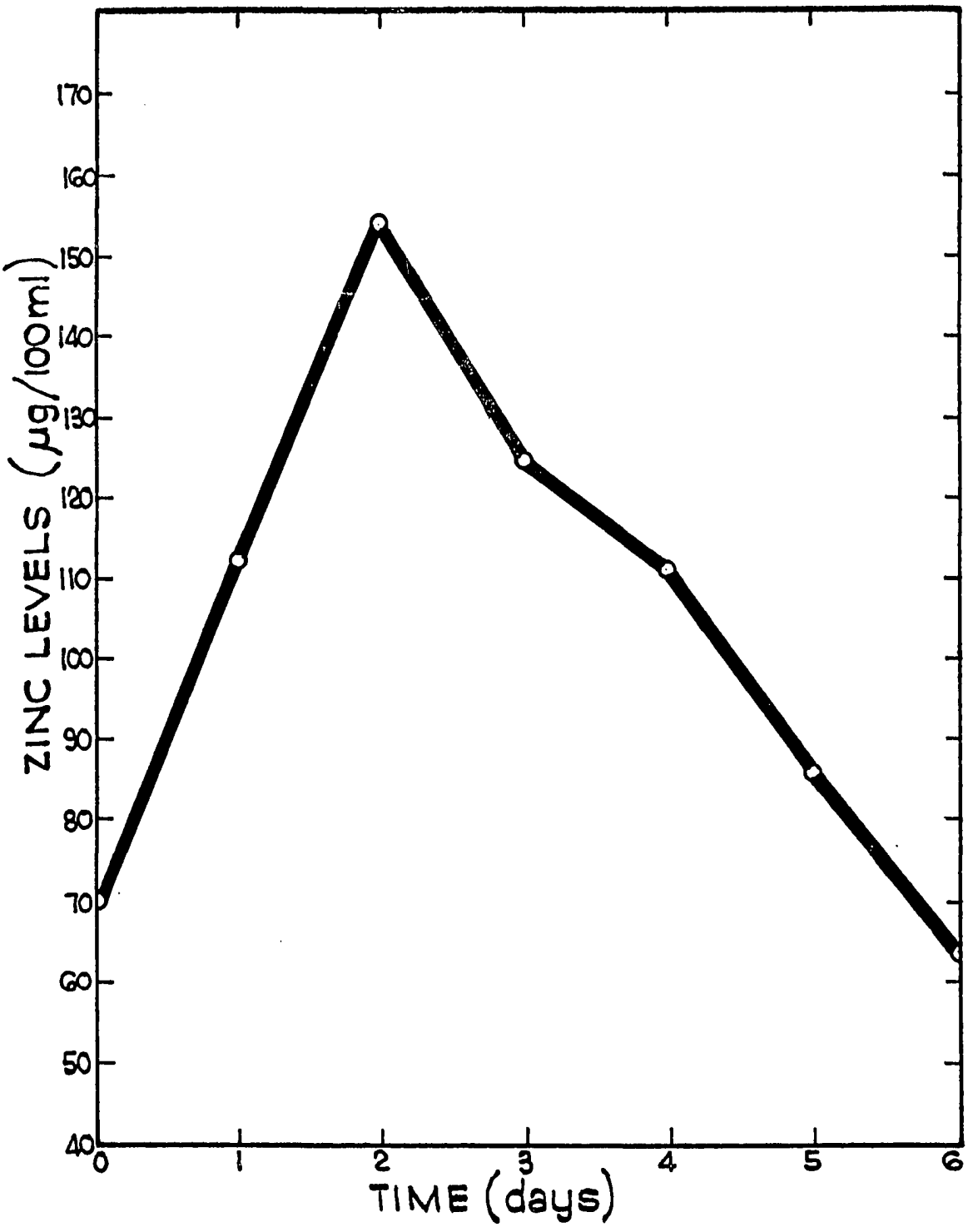


Figure 3. Average plasma zinc level from the daily dog study.

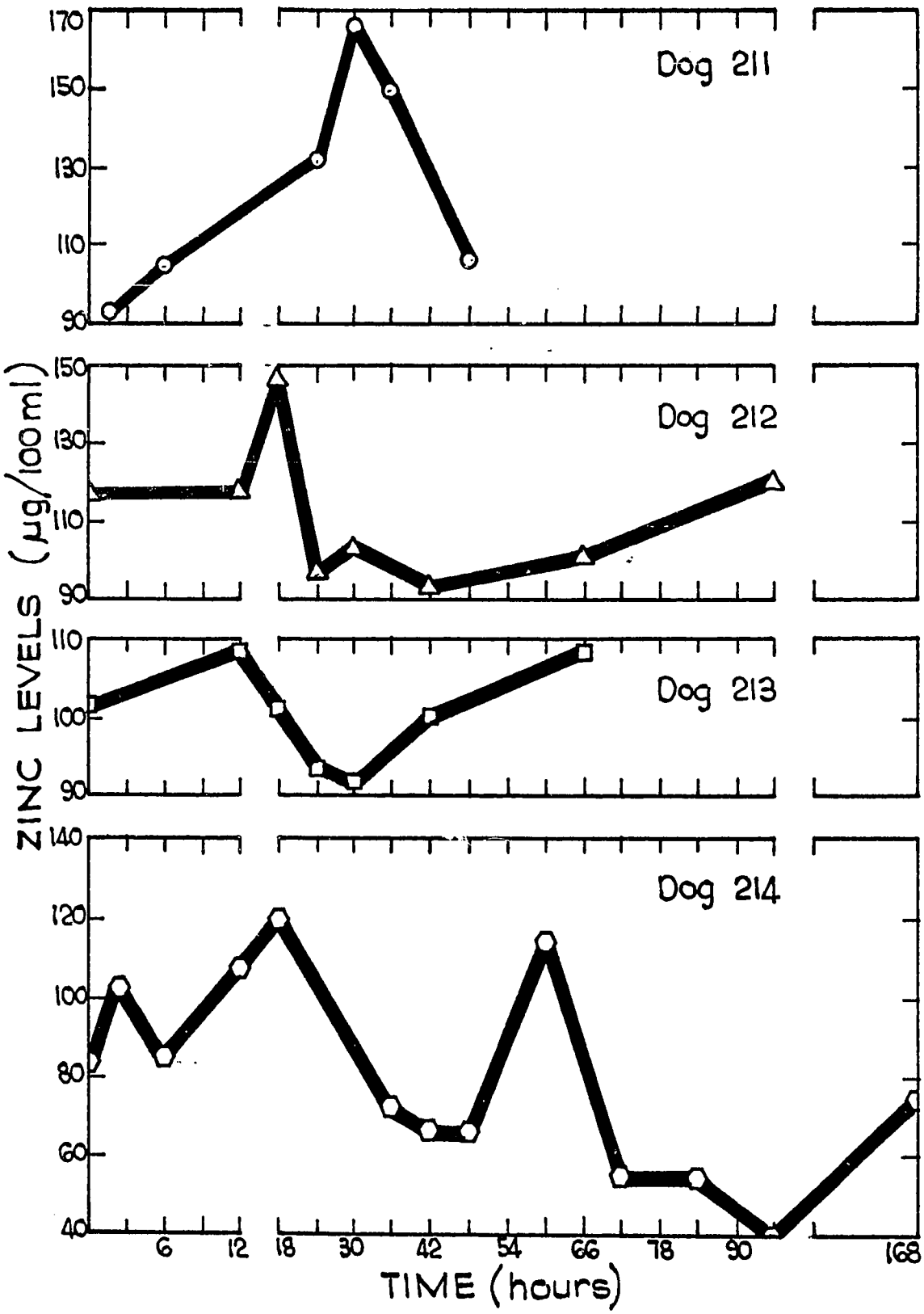


Figure 4. Wound plasma zinc levels from the hourly dog study.

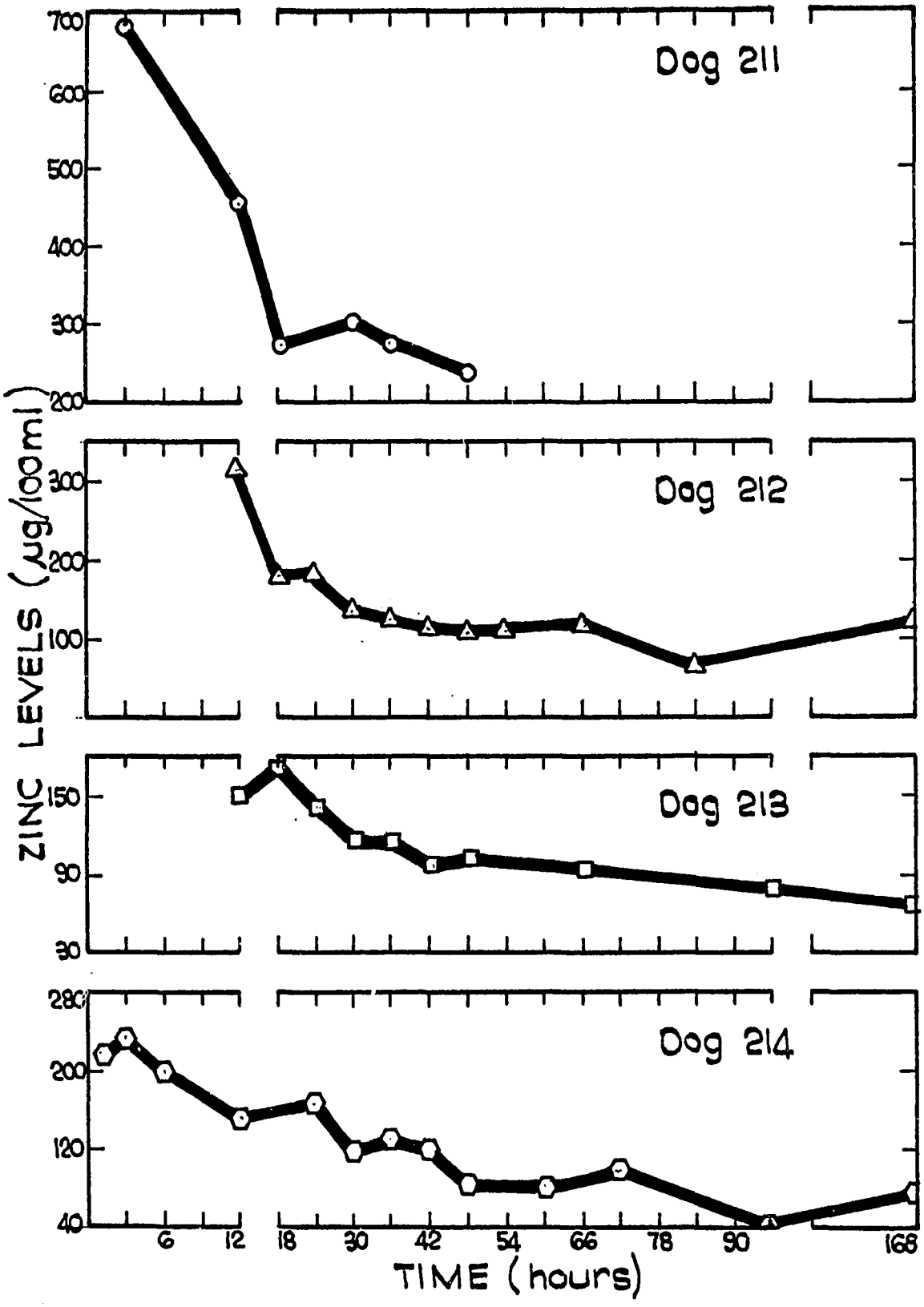


Figure 5. Wound fluid zinc levels from the hourly dog study.

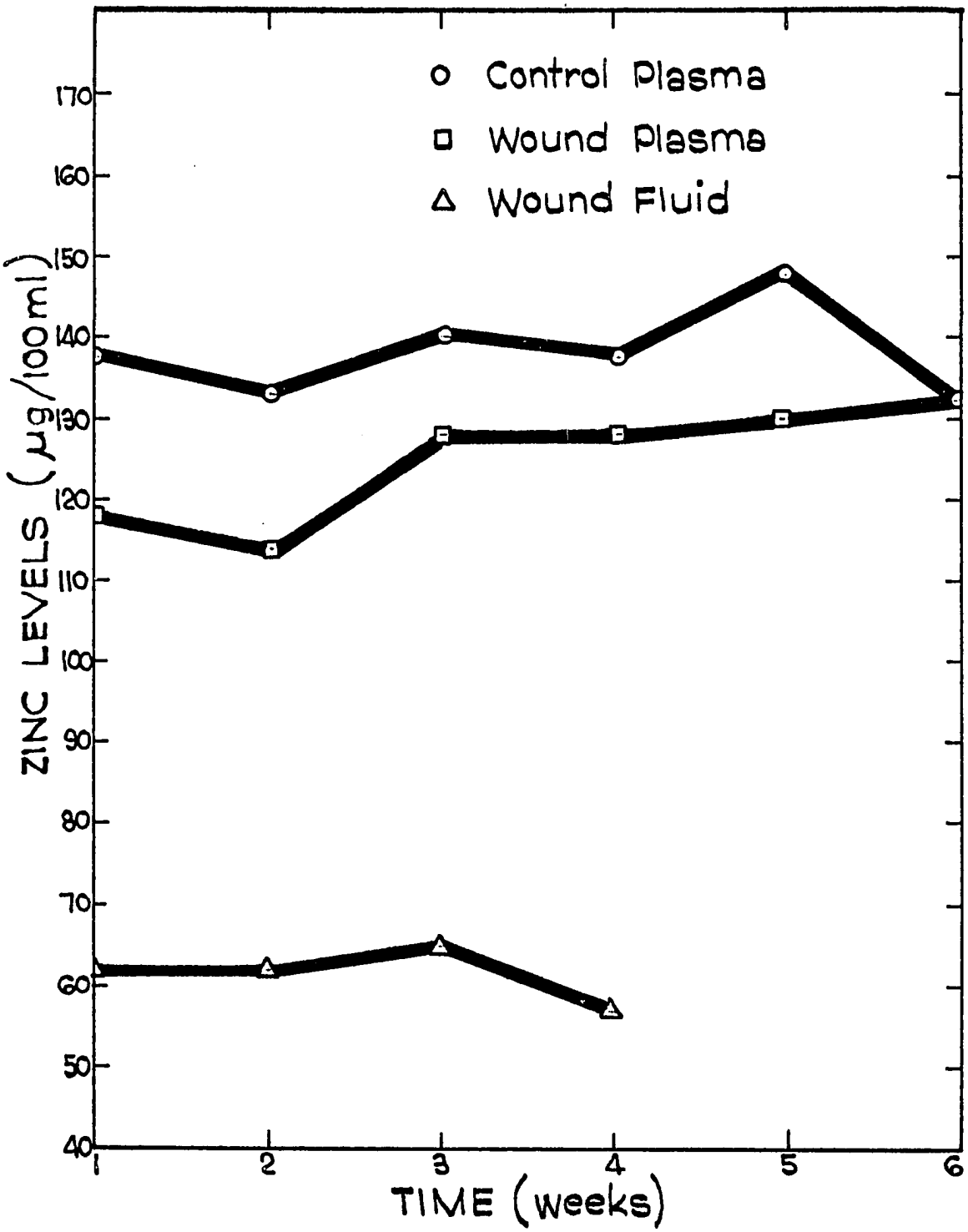


Figure 6. Average control plasma, wound plasma, and wound fluid zinc levels from the weekly rat study.

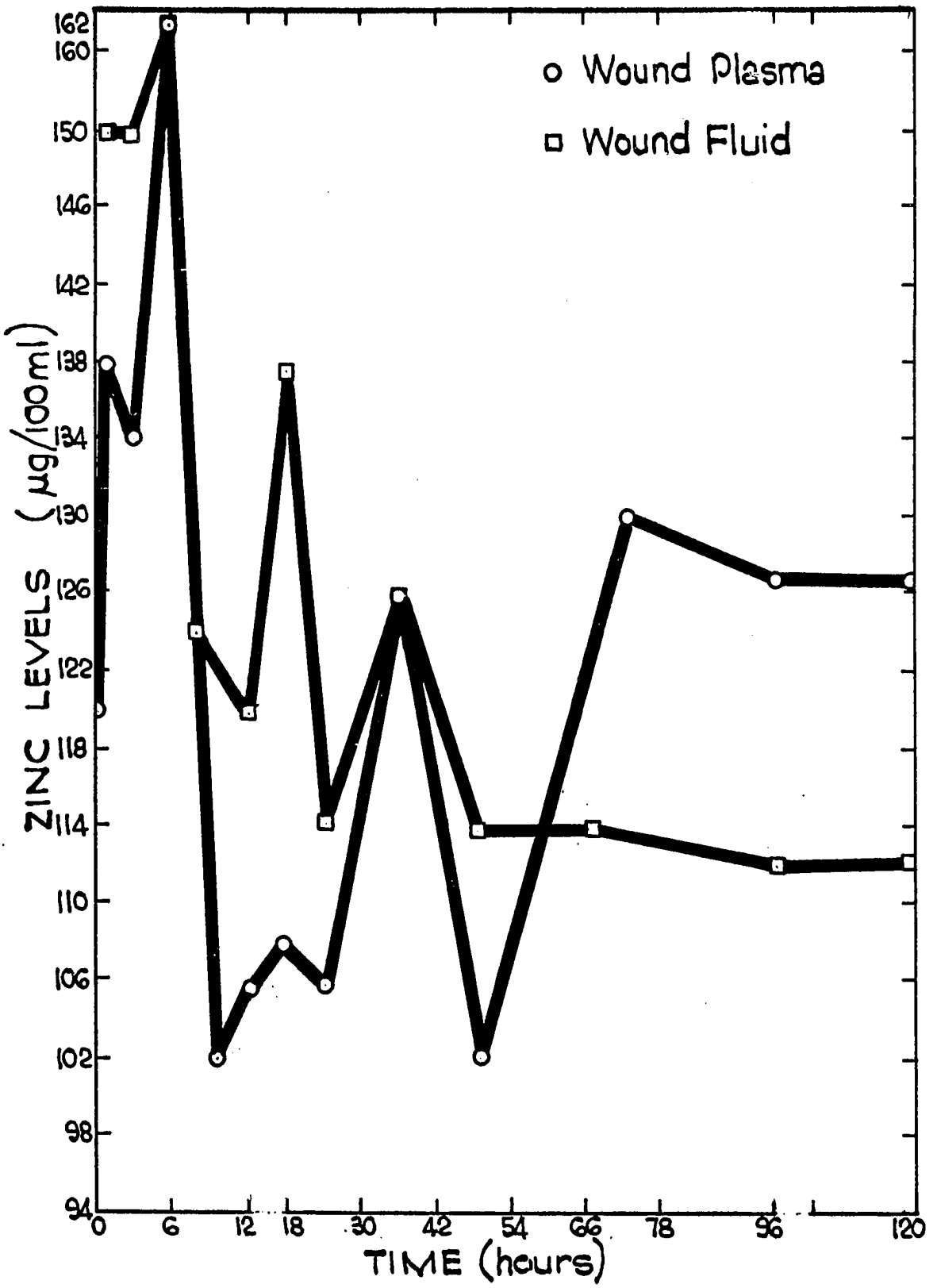


Figure 7. Average wound plasma and wound fluid zinc levels from the hourly rat study.