CHROMATES AND ANIMAL NUTRITION

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By

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PREFACE

The increasing industrial uses for chromate salts in pigments and chromic acid in plating baths present a problem of toxicity if industrial wastes are allowed to contaminate neighboring streams. It has been shown that neutral potassium chromate in quantities as small as 1.9 grams is lethal to rabbits in two hours. Subcutaneous injections of .2 to .4 gram of potassium chromate were found by Gergens and Posner (5) to act with great intensity on rabbits, death often occuring within a few hours. Other studies of the toxicity of chromates have been concerned largely with inhaled, subcutaneous, and intravenous poisonings.

In view of this, the determination of the level of chromate salts that an animal can ingest without injury or impairment of general health and reproduction makes such an investigation necessary.

The largest use of chromates in pigments is as the zinc salt which does not darken on exposure to sulfides as lead salts do. Thus it seemed advisable to include zinc chromate in this toxicity study.

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INTRODUCTION

A case of asthma is described by Card (3) which was caused by five months' exposure to the fumes and dust of a chromium-plating factory. An attack could be produced artificially by intradermal injection of four milligrams of potassium chromate or dichromate. Graham (7) reported a patient engaged in chromium-plating for two and a half years developed an excessively troublesome cough and hoarseness. There was also loss of sleep and weight with anorexia, fatigue, and bleeding from the nose. The nasal septum was perforated. Removal from the fumes, inhalation of Friar's balsam, free elimination, together with an iron tonic, resulted in complete recovery. Alwens and Jonas (1) report recently established cases of lung cancer in chromate workers, most of whom had long since left the industry. The latency period was sometimes as long as thirty years. Chemical investigation revealed a distinct storage of chromates in the lung tissue. The chronic injury of the nasopharynx through the continuous irritation of the chromates and other industrial products was a particularly marked factor. The hilus glands were always enlarged.

Other cases of subcutaneous nature are as follows: Major (10) stated in a case history that after cauterization of a wound with chromic oxide, a nephritis promptly developed, with death resulting in thirty days. The kidney lesion was that of a pure tublular nephritis. There was no edema, ascites or anasarca, or any symptoms of uremia. The patient felt well. The excretion of urine was almost completely suppressed after two days, but later increased to 1,700 milliliters; then fell again a few days before death.

Goertz (6) reported: In the chrome process for the control of concretions, rust, and corrosion in heating plants, the boiler is first flushed with a cold solution and rinsed. The chromium solution is then added to the circulating water to dissolve the lime deposits and incrustations in the pipes. The hands, faces, and clothing of two workmen were accidentally sprayed with this solution. Severe burns, particularly on the face, combined with symptoms of very severe chromic acid poisoning terminated fatally for one of them in two weeks. Great care is necessary in the transfer of the chrome solution, since the chromic acid can enter the body through very small wounds and cause death by poisoning of the kidneys and lungs. Yet, he stated that the industrial uses of chromic acid and chromates do not cause kidney damage. Fatal kidney damage occurs, however, upon ingestion of these substances. Since, in the cases described, erosions appeared on the lips and nasal orifice, he assumed that the chromic acid was not adsorbed through the skin, but that some of it had been swallowed.

Roels (14) found that oxygen and hydrogen are liberated which atomize the chromic acid from the bath. The acid damages the mucous membranes and may eat through the cartilagious septum. Chromic acid produces on the skin indolent and painful sores called "chrome holes".

The pharmacologic study of chromate and dichromate salts by Rabbeno (13) revealed that the immediate intravenous minimum lethal dose of potassium dichromate, sodium dichromate, and sodium chromate at a velocity of injection of .00006 gram-moles per kilogram per minute is .0009, .00175, and .00232 gram-moles respectively, or a relative toxicity of 1:.5 :.4. The toxicity curves show that, within the limits

of this experiment, increasing the velocity of injection increases the dose necessary to be toxic to the rabbits--a behavior opposite to that usually shown by drugs. The dose seems to be independent of the veloc- of injection and dependent only on time. The methemoglobin formed during the injection of a .123 molar solution of sodium dichromate follows an S-shaped curve, and the maximum amount found at death varies from 58.5 to 97.75 percent of the total hemoglobin. This is independent of the velocity of injection and of the dose injected.

Brieger (2) reported that the injection of chromic acid and chromate salts is followed by uremic symptoms, severe necrosis of the epithelium of the kidneys, leucocytic and myeloid changes in the bone marrow. The intensity of the reaction is independent of the chromium content.

Ophuls (12) found it was impossible to produce severe lasting renal lesions in guinea pigs with chromate salts, possibly because in these animals the chromate salts are too toxic in a general way and too slightly effective on the kidney locally. Also, he found that the acute renal lesions produced in rabbits by large sublethal doses of chromate salts are much more marked than those found in guinea pigs under similar conditions. There was marked albuminuria, much degeneration, necrosis and desquamation of the epithelium, and abundant formation of casts of different kinds.

Sander and Camp (15) reported a case of chromate poisoning in an infant owing to the ingestion of paint containing a relatively insoluble chromate compound. There was evidence of local gastro-intestinal irritation. The symptoms were systemic and showed evidence of reaction on the nervous system. Chromium was found in the urine and feces.

The toxicity of zinc has been investigated by Heller and Burke (8) and the metabolism of zinc has been investigated by others. Drinker, Fehnel, and March (4) presented figures upon the normal amounts of zinc present in the urine and feces of a large group of healthy adults on an ordinary mixed diet. McCance and Widdowson (11) carried out forty-five zinc balances on normal persons and patients. Within the limits of experimental error, normal adults excreted almost the same amount of zinc in their feces as they ingested in their food.

Sheline, Chaikoff, Jones, and Montgomery (16) studied the excretion of intravenously injected radio-zinc in the urine and feces of dogs and mice. The results they obtained showed that a large fraction of the body zinc is eliminated by way of the gastro-intestinal tract.

EXPERIMENTAL

In order to determine the highest level at which animals can tolerate chromate salts in their drinking water or food without restraining growth, affecting physical characteristics, or showing other deleterious effects, white mice, white rats, and albino rabbits were chosen as experimental animals. They were used because of the ease of handling, permitting the observation of large numbers under various conditions, and because it was of interest to know not only the temporary conditions but also the effect on reproduction and the possibility of sterility that might be produced by the accumulation of the metal in the bodies of the animals.

The basal ration used was known to produce satisfactory results for growth, reproduction, and rearing of young in this laboratory. Mature mice were placed in cages so that each lot would be comparable so far as possible at the beginning of the experiment. Each cage of animals received different amounts of chromate salt in its drinking water--100, 200, 300, 400, and 500 parts per million of neutral potassium chromate; while the sixth cage of mice received one percent zinc chromate in its feed and ordinary tap water to drink. All remained healthy and reproduced normally.

To find the influence of chromates on growing animals, young white rats were placed four in a cage. Cage 1 received the basal ration and drinking water containing 300 parts per million potassium chromate. Cage 2 received the basal ration and drinking water containing 500 parts per million potassium chromate. The basal ration of Cage 3 contained

the addition of one percent zinc chromate and the drinking water was ordinary tap water. Controls were carried along at the same time on the basal ration and tap water. Neutral potassium chromate was added to the feed of four other cages of rats. Cage 5 received one-eighth of one percent potassium chromate; Cage 6 received one-fourth of one percent potassium chromate; Cage 7 received one-half of one percent potassium chromate; and Cage 8 received one percent potassium chromate. All animals in this last group received tap water. The animals were weighed once a week and their growth curves plotted by months (Charts 1 to 9). The first two months of the growth curve are the most important because growth is the most rapid during this period.

These curves show that the highest level of chromates which can be tolerated are as follows: 500 parts per million potassium chromate in the drinking water and one percent zinc chromate in feed. These figures are for animals half-grown or older only. Younger animals receiving zinc chromate were stunted by very small amounts. The lowering of digestability of the feed (Charts 10 and 11) is a possible explanation. The level of tolerance for potassium chromate in feed is one-eighth of one percent of the total ration.

In order to determine the path of elimination of the chromium compounds, two metabolism cages containing three white rats each were used. The occupants of the first cage were fed the basal ration and their water contained 500 parts per million potassium chromate; the animals in the second cage received one percent zinc chromate in their feed and tap water. The separated feces and urine were ashed and dissolved in dilute sulfuric acid; the silica was filtered out and the filtrate analyzed

qualitatively for chromate by the colorimetric method of Yoe (18). (Method is described in appendix of this paper.) This test was found to be extremely sensitive, since .0000001 gram of chromium gave a quantitative reading at 535 millimicrons (optical density .06) in the Coleman Universal Spectrophotometer, Model 11. The test on the feces was positive and the test on the urine was negative showing that the path of elimination was entirely through the intestines. This was later confirmed when the digestive coefficients were determined using rabbits as experimental animals. The blood of the rabbits was also tested for chromium. Five milliliters of blood were drawn at the end of each of the three digestive trials from the rabbit receiving 500 parts per million of potassium chromate and from the one receiving zinc chromate. These samples were ashed and the same test for chromium was run. All analyses gave a negative result, showing that there was no chromium in the blood stream. This indicated that the chromates did not enter into the blood from the alimentary canal; otherwise traces of chromate would have been found both in the blood and in the urine.

Three mature young female rabbits were placed in metabolism cages for the digestive coefficient trials. Their basal ration had the following analysis: moisture 8.91%, ash 6.93%, fat 3.21%, crude fiber 7.48%, nitrogen-free-extract 54.69%, calcium .406%, and phosphorous .456%. Rabbit 1 received the basal ration and tap water; Rabbit 2 received the basal ration and 500 parts per million potassium chromate in its drinking water; and Rabbit 3 received tap water and the basal ration with one percent zinc chromate added to it. After allowing a week for them to become accustomed to the feed and their new surroundings, the trials began.

Collections were made daily over three periods of seven days each. During these periods the quantity of feed consumed was weighed, and the urine and feces were collected separately by the use of a false screen floor. The feces were preserved by rapid air drying, and the urine samples were preserved with sulfuric acid to keep at a minimum enzymatic action and to stabilize the nitrogen. Sufficient acid was used to keep the urine acidic at all times. Ten milliliters of the concentrated acid was necessary each period. At the end of the experimental period, the feces were ground and the urine was diluted to a definite volume with distilled water. Feces and feed were analyzed quantitatively for moisture, ash, total nitrogen, fat, fiber, nitrogen-free-extract, calcium, and phosphorous; and the urine for total nitrogen, calcium, phosphorous, and ash according to the latest methods of the A. O. A. C.* From the analytical data food digestibility values were calculated, and were used in conjunction with growth and reproduction records and blood analyses as criteria of the nutritional value of the basal ration under different conditions. (Digestive coefficients and other nutritional data are in Tables 10, 11, and 12. The discussion of the digestive trials is in the following section.)

A partial explanation of the negative results of the test for chromates in blood and urine samples was determined by the following experiment. Fifty grams of dried feces (from rabbit drinking water containing 500 parts per million potassium chromate) were extracted with water overnight in a Soxlet Extractor. The filtrate was given both the diphenylcarbazide test (see Appendix) and the barium chloride test which would

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*Association of Official Agricultural Chemists

have given a bright yellow precipitate if ionic chromates were present. Both tests were negative. This showed that there were no water-soluble chromates in ionic form. When this filtrate was evaporated to dryness, ashed, and re-oxidized to the chromate ion, a very strong positive test was obtained with no difficulty. A plausible explanation is that proteins are known to be coagulated by chromates, forming an insoluble precipitate. This would have prevented a positive test. However, repeated extractions in the Soxlet apparatus must have peptized this complex and allowed it to pass into the filtrate with the other water-soluble compounds in the feces. Conclusive evidence as to what actually happens in such a complex media as the digestive tract of a living animal would be practically impossible to obtain.

DISCUSSION OF DIGESTIVE TRIALS

The apparent digestion coefficients of nitrogen, ash, calcium, phosphorous, and fat are comparatively consistent. Rabbit 1, the control, and Rabbit 2, the animal which received 500 parts per million potassium chromate in its water, digested the feed comparatively the same, the difference between the two being insignificant. Rabbit 3, whose basal ration contained the addition of one percent zinc chromate, had lower digestive coefficients for nitrogen, ash (expected since an insoluble salt had been added to the feed), calcium, phosphorous, and fat. All were significantly below normal, showing that zinc chromate does interfere with digestion.

The crude fiber digestion was increased by the presence of potassium chromate and tremendously decreased by the presence of zinc chromate. The coefficients for nitrogen-free-extract were almost identical for all three animals.

CONCLUSIONS

(1). The toxic level of potassium chromate in water and the toxic levels of insoluble and water-soluble chromate salts in feed have been determined for mice, rats, and rabbits.

(2). Growth curves of white rats eating and drinking feed and water contaminated with chromate salts show that the maximum non-toxic levels are 500 parts per million of soluble chromate in water; one percent zinc chromate in feed, for mature animals only; and one-eighth of one percent potassium chromate in feed.

(3). The path of chromates through the digestive tract is unlike other soluble salts and does not agree with a report in the literature.

(4). Digestive trials show that contaminated drinking water up to 500 parts per million does not affect the utilization of food by the animal and show that one percent zinc chromate has a marked lowering of digestive coefficients on practically all components of the feed.

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APPENDIX

Colorimetric determination of chromium: (As described by Yoe (18)).

The observation of Cazeneuve that diphenylsemicarbazide and chromic acid give a purple-colored solution* has been applied to the colorimetric determination of chromium. The chromium is first converted into chromate by oxidation with sodium peroxide and acidified with sulfuric acid. Dissolve ashed sample in sulfuric acid; when solution is complete add nitric acid and heat until dense fumes are evolved. Cool, dilute with a little water; add sodium hydroxide solution and sodium peroxide; and boil until the excess peroxide is destroyed. Cool; dilute to volume; and to an aliquot, add diphenylsemicarbazide reagent and sulfuric acid. An intense purple-colored solution is formed which is read in the spectrophotometer.

Metabolism Cages: A metabolism cage is an animal cage with a hole slightly larger than the animal's head in one end of it to permit the animal to eat the feed in another much smaller cage. The floor of the main cage is a very coarse wire screen which allows the feces and urine to pass through it. A few inches below this false floor is another screen of a rather fine mesh which collects the feces. The entire cage rests on a large funnel which allows the urine to pass into a container beneath it. Thus the feces are not contaminated with feed, or the urine with feces, bringing about complete separation of feed spilled by the animal and the feces and urine.

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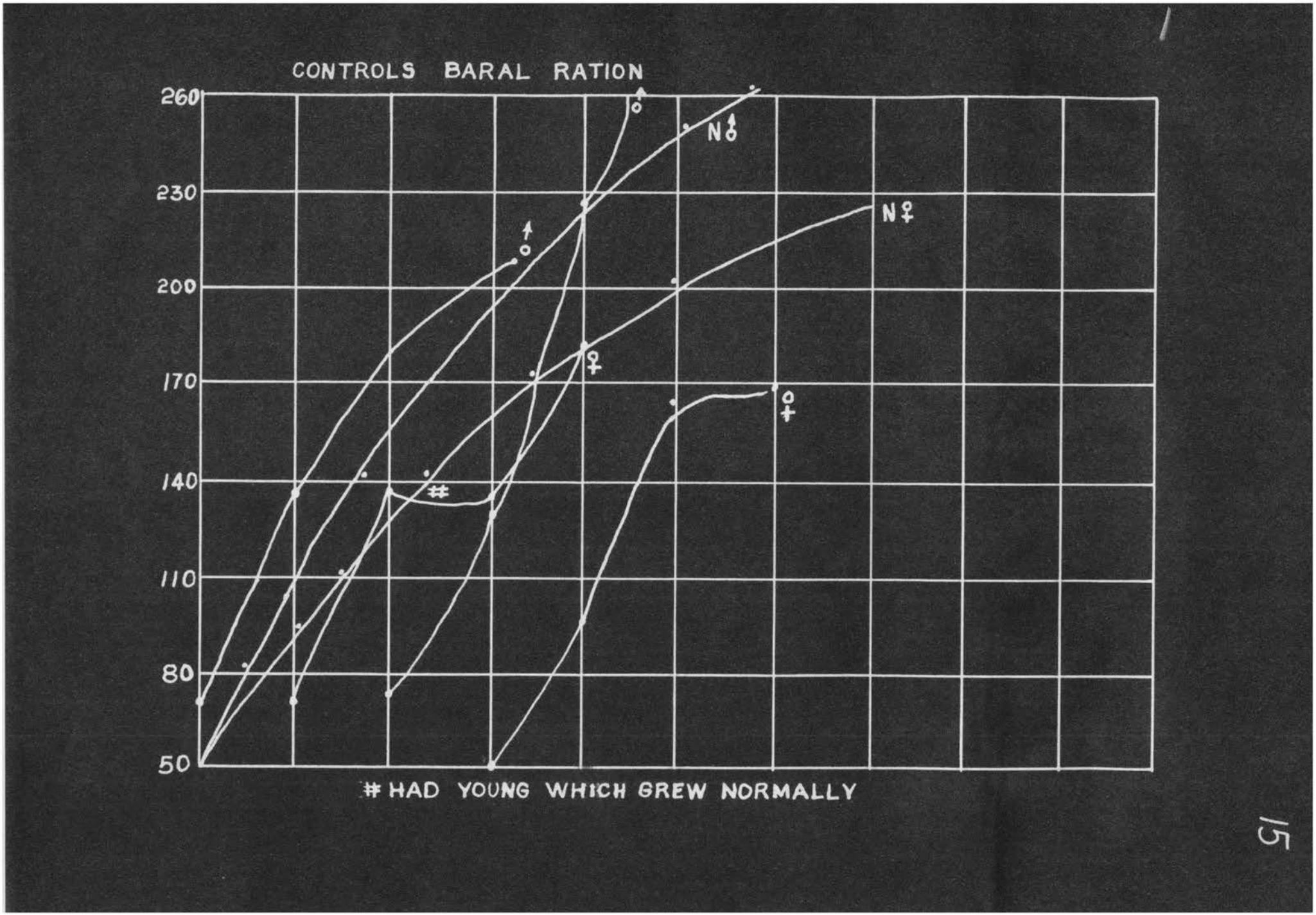
*This reaction has also been used by Herrman and Lederle (9) for the determination of traces of chromium in soil because this test is very sensitive and specific for chromium under these conditions.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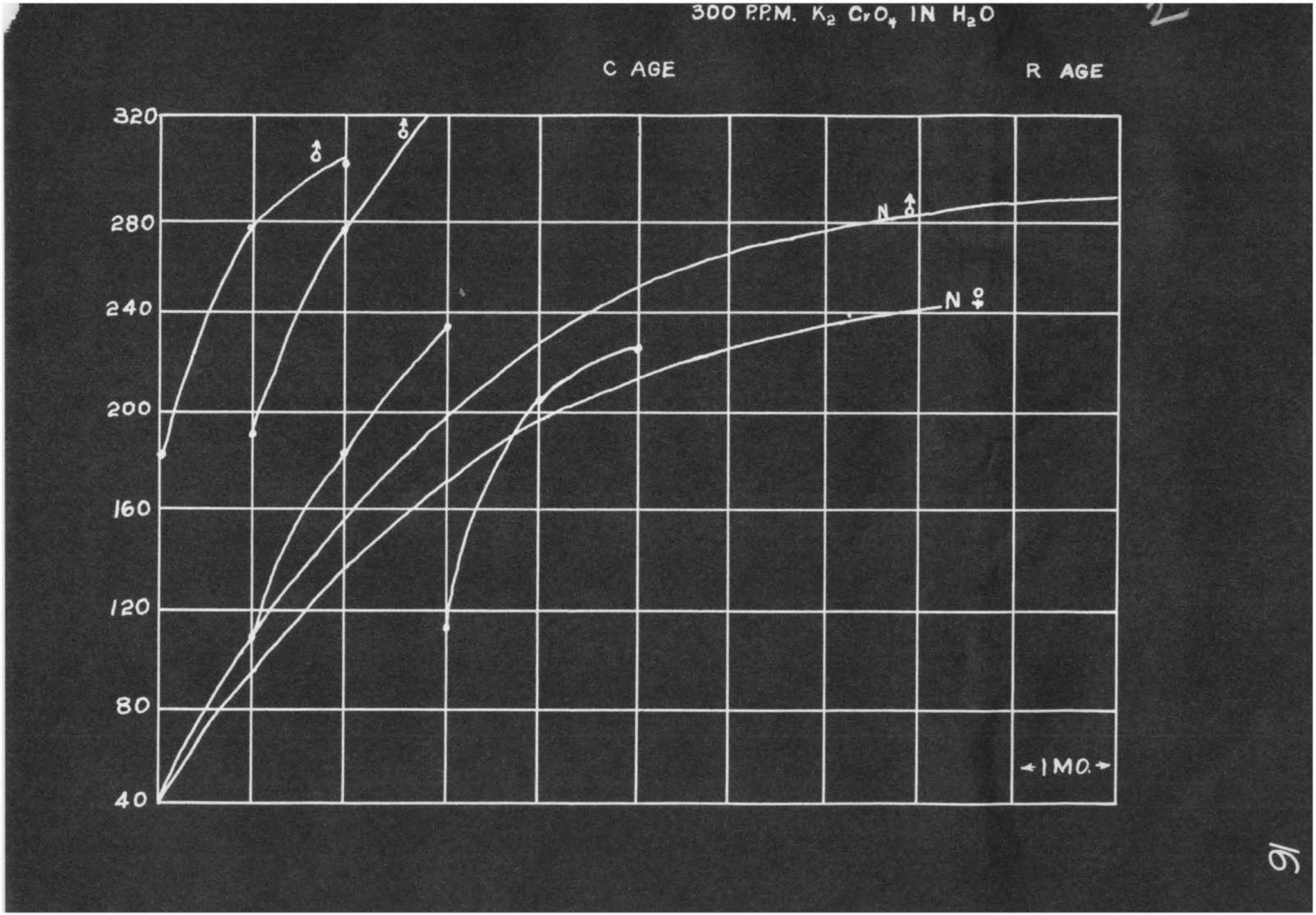
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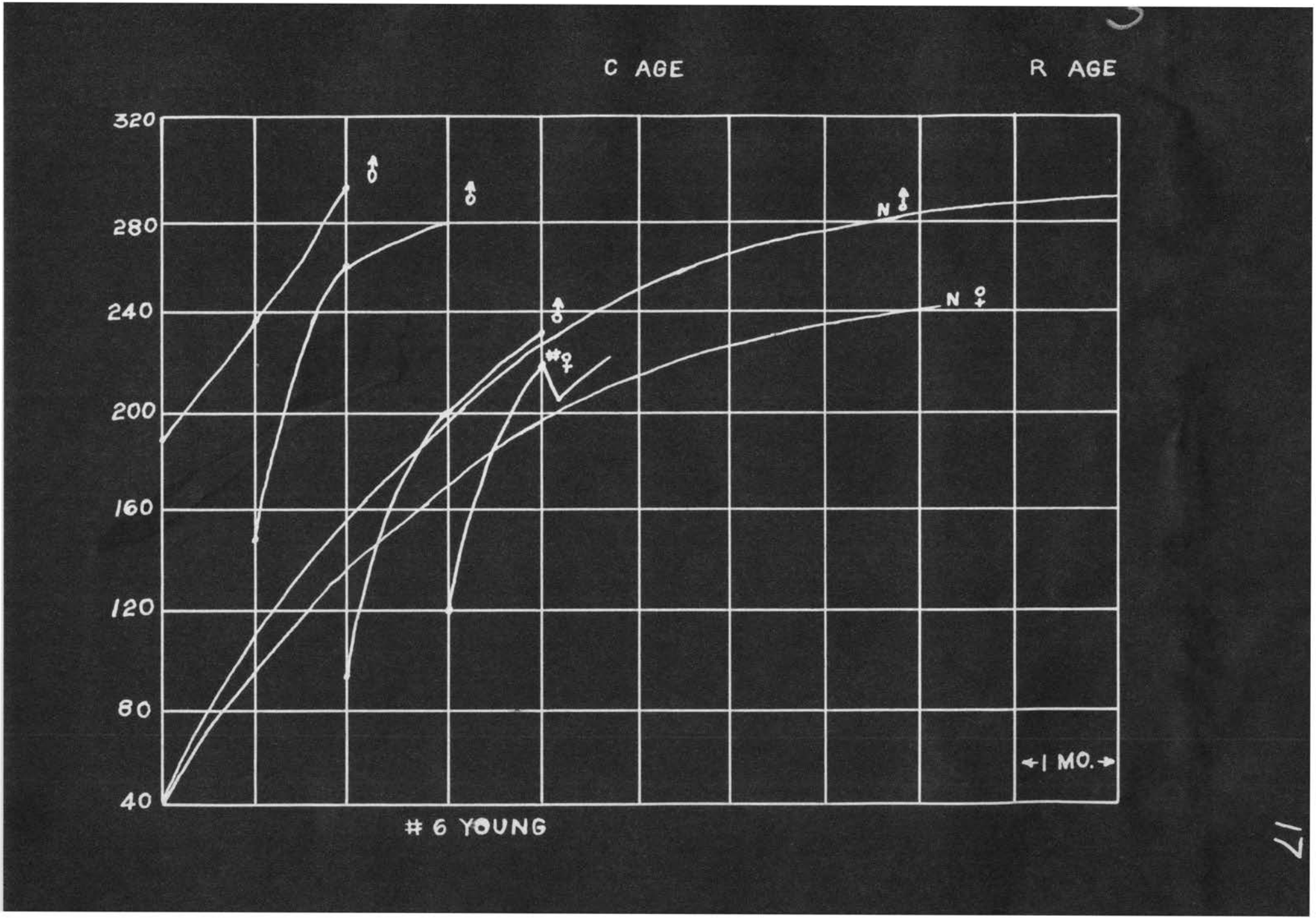
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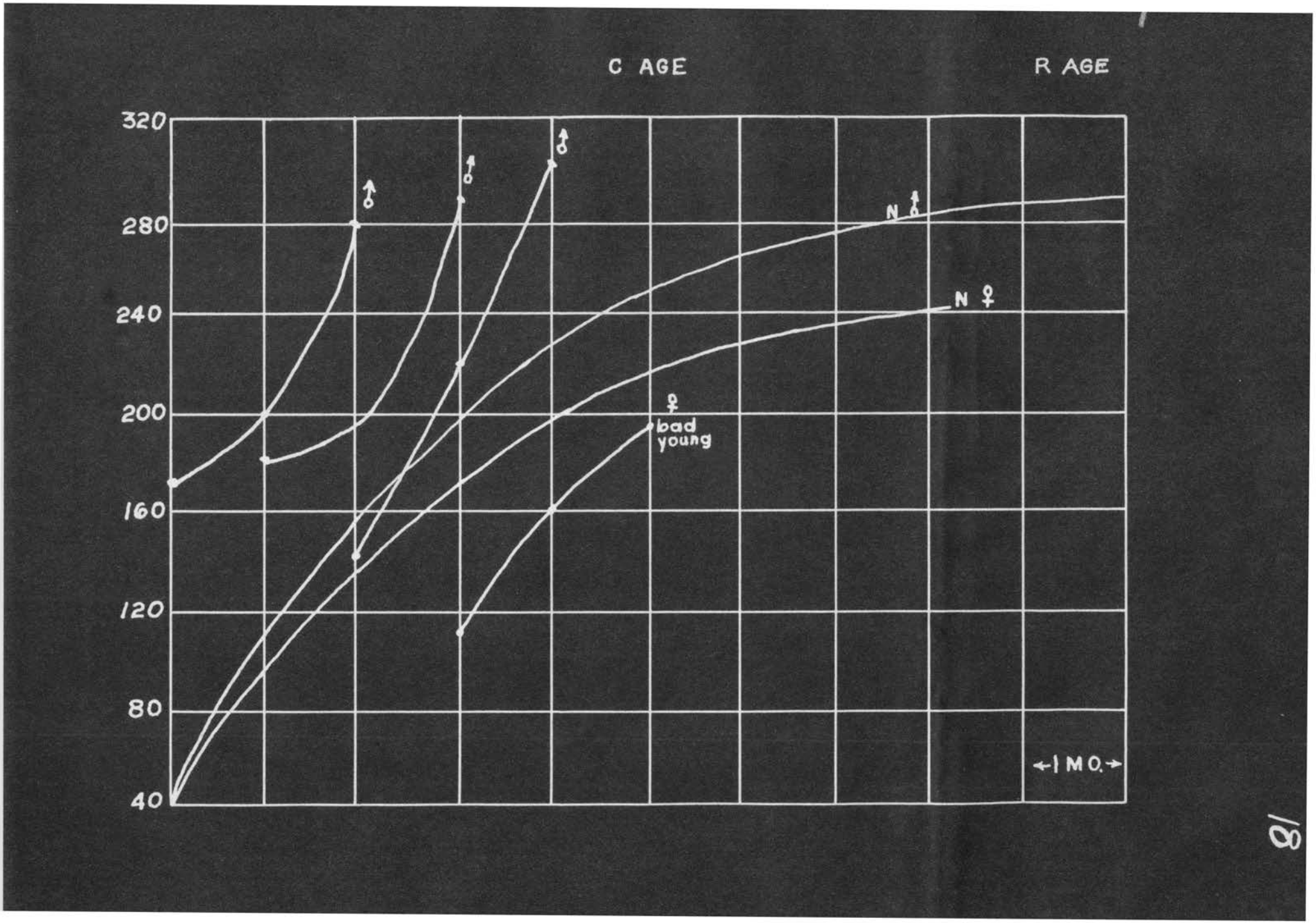
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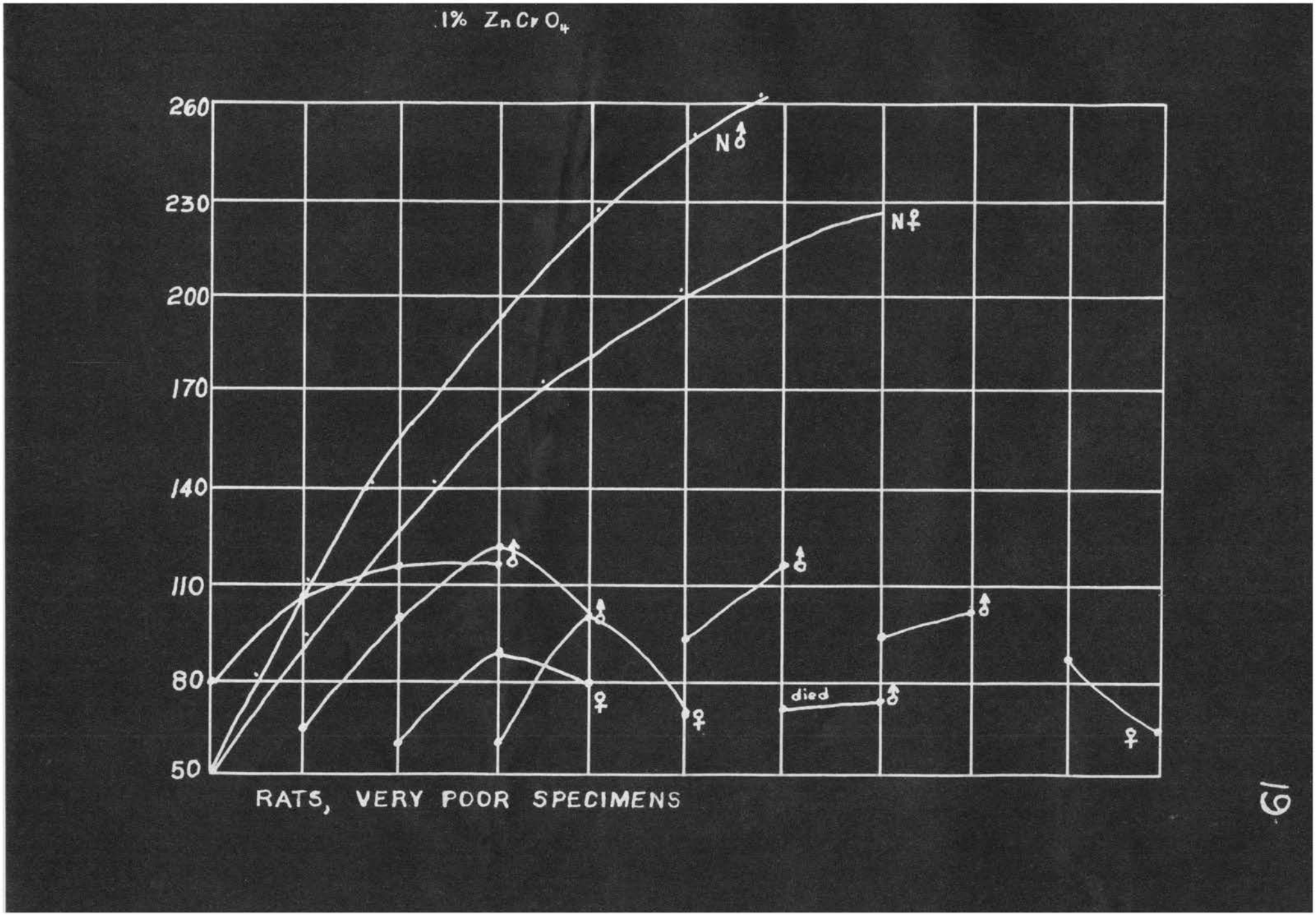
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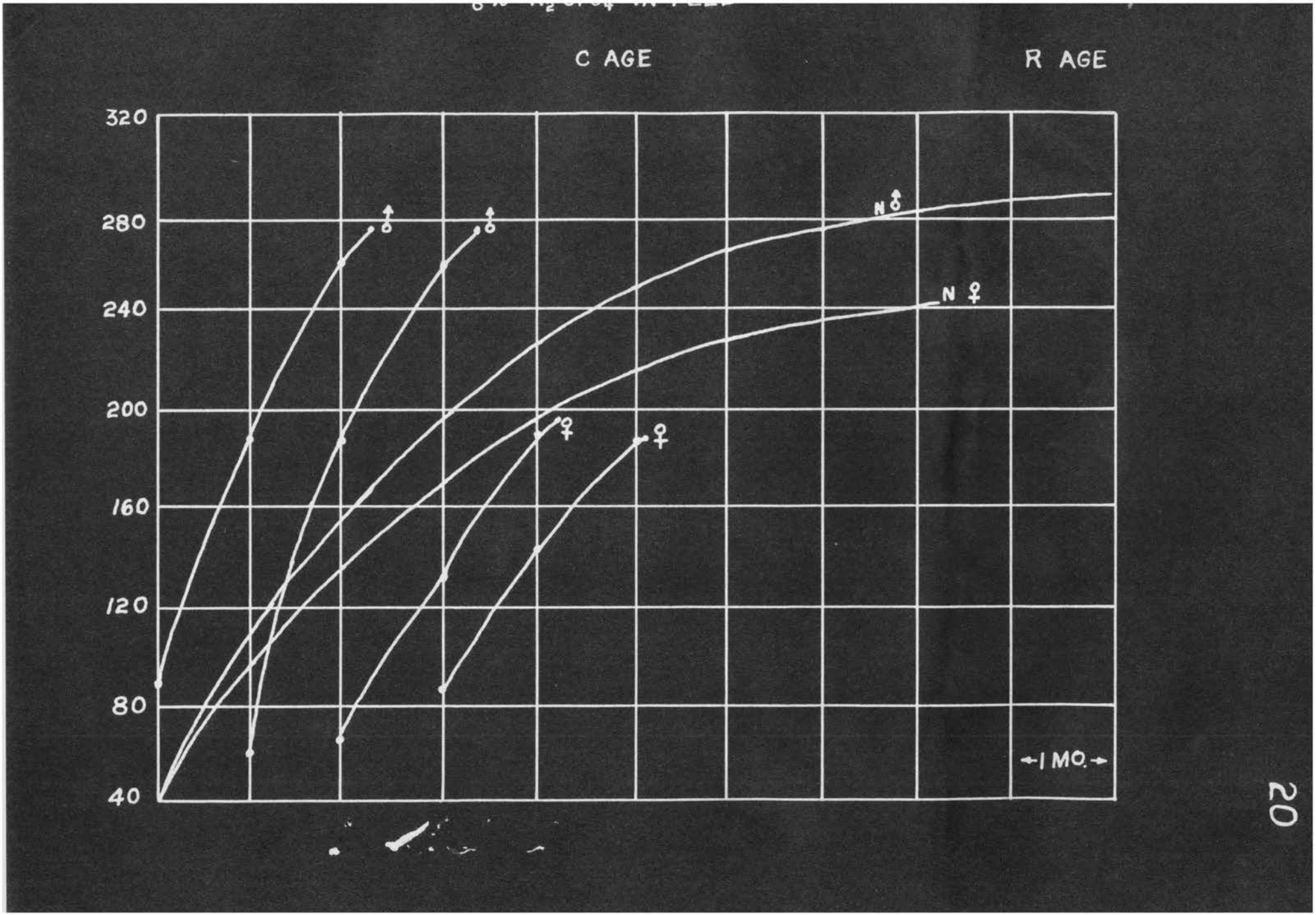
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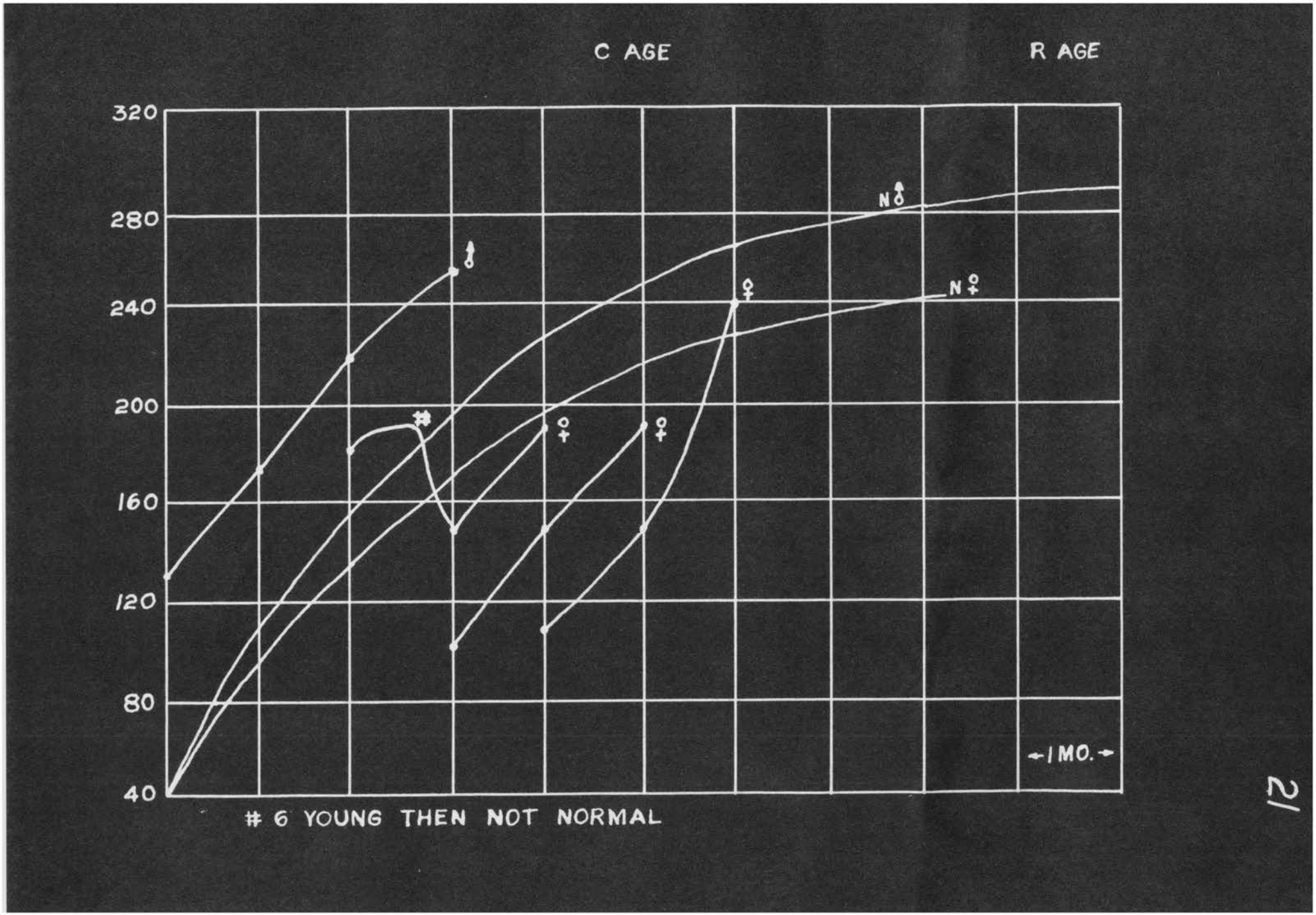
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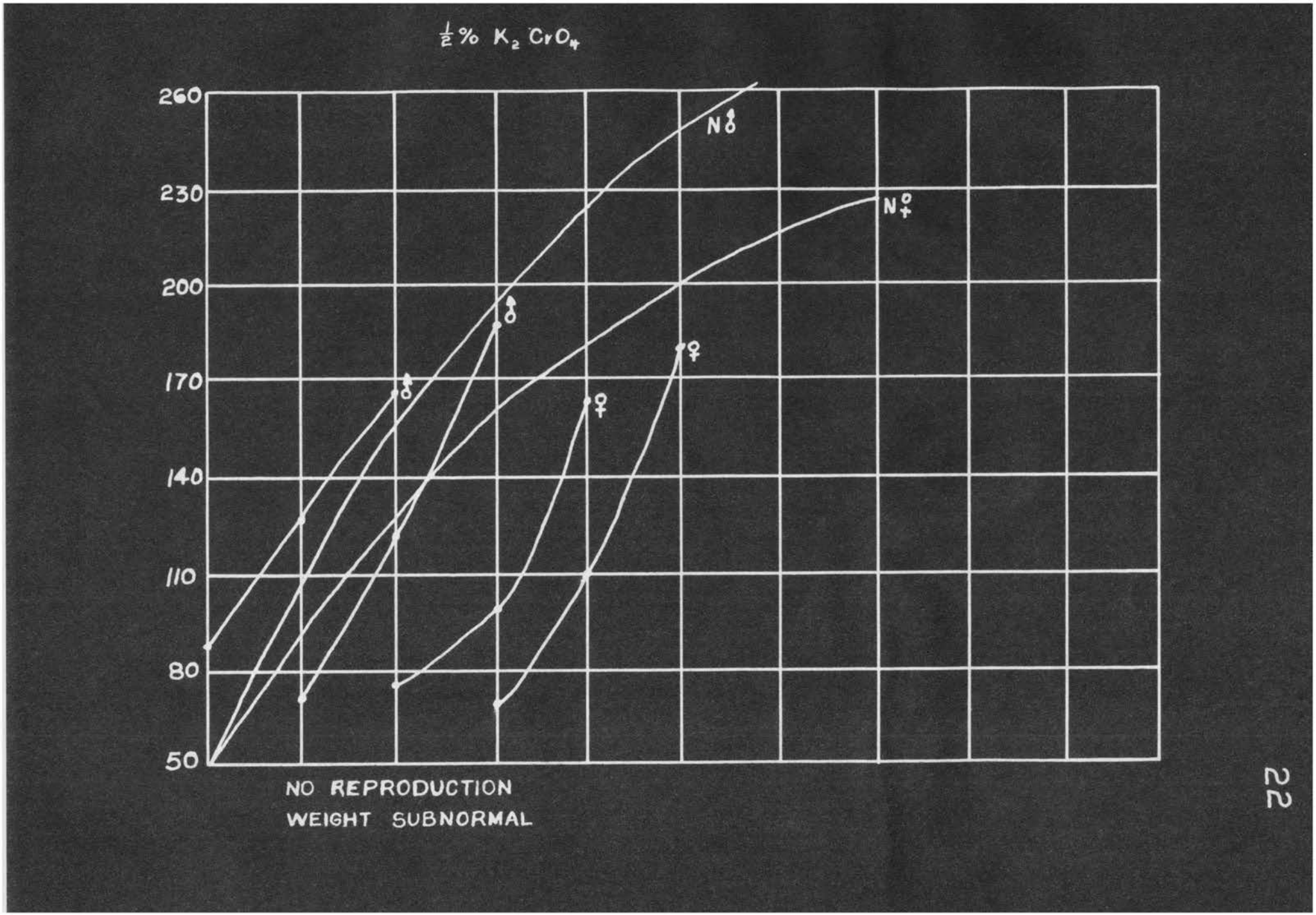
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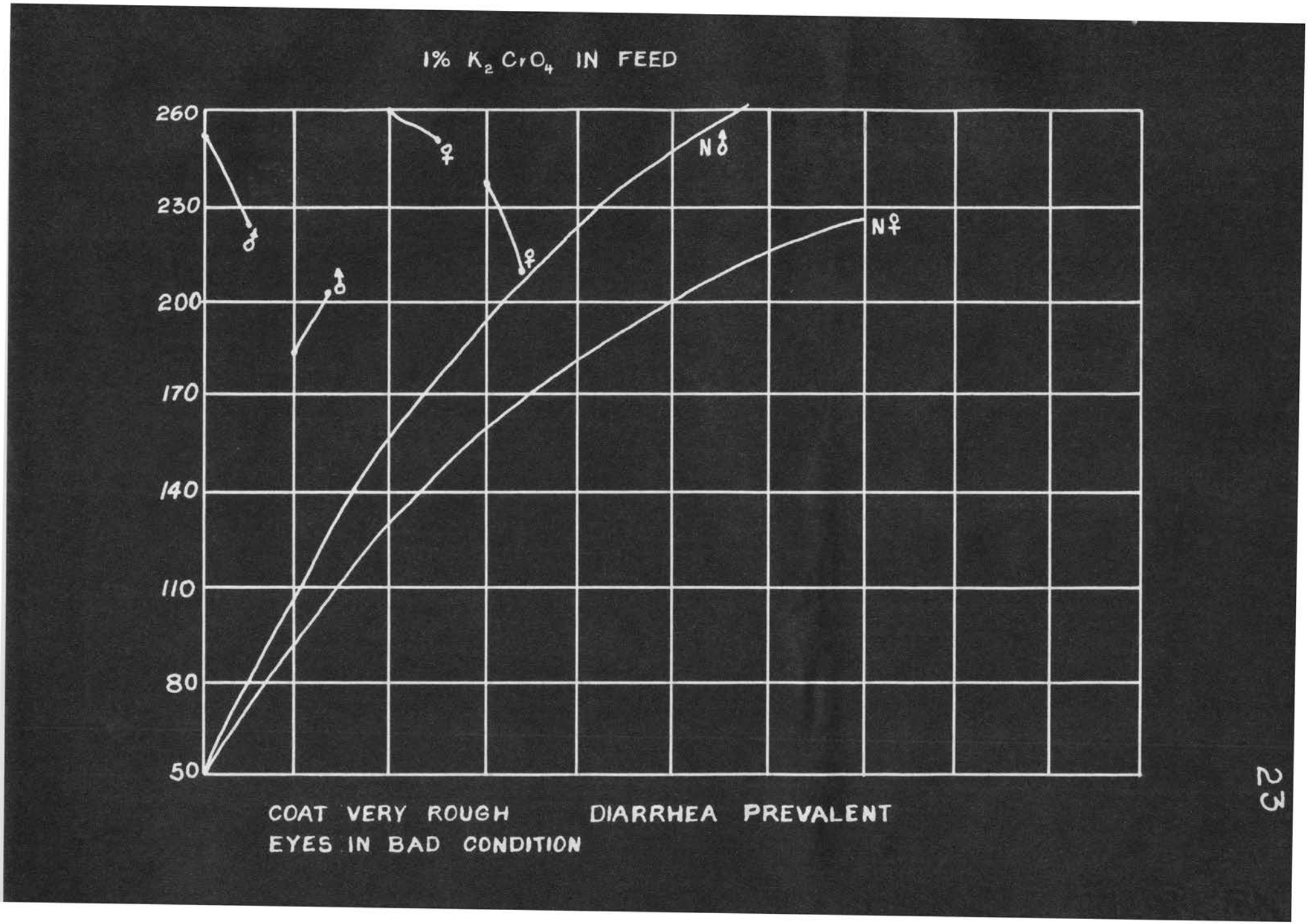
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COAT VERY ROUGH
EYES IN BAD CONDITION
DIARRHEA PREVALENT

DIGESTIVE DATA

Period		Grams Eaten	Per cent	Grams per Period	Grams of Feces	Per cent	Grams per Period	Milliliters of Urine	Milligrams per Milliliter	Grams per Period	Grams retained	Per cent Retained	Per cent in Feces	Per cent in Urine	Digestive Coefficient
NITROGEN BALANCE															
Rabbit	I	262	3.005	7.8	33	3.185	1.05	800	7.31	5.85	.86	11.54	13.46	75.00	86.54
Control	II	337	3.005	10.1	69	2.210	1.52	1100	5.225	6.30	2.28	22.87	15.05	62.38	84.95
	III	522	3.005	15.7	129	2.586	3.34	1100	6.96	7.66	4.70	29.94	21.27	48.79	78.73
K_2CrO_4	I	198	3.005	5.95	35	1.855	.65	525	11.225	5.89	-.61	-9.62	10.92	99.00	89.08
(500p.p.m.	II	286	3.005	8.59	54	2.020	1.09	450	13.70	6.16	1.34	15.61	12.68	71.71	87.32
in water)	III	449	3.005	13.49	85	2.194	1.86	600	12.90	7.74	3.89	28.83	13.79	57.38	86.21
$ZnCrO_4$	I	307	3.295	10.10	15.5	1.405	.218	620	6.97	4.32	5.64	55.84	2.16	42.77	97.84
(1%) in	II	204	3.295	6.71	56.5	2.559	1.446	500	8.60	4.30	.96	14.30	21.55	64.08	78.45
feed	III	264	3.295	8.69	70.0	2.716	1.901	500	5.99	3.00	3.79	43.61	21.86	34.52	78.14
ASH BALANCE															
Control	I	262	6.93	18.16	33	11.76	3.88	800	16.74	13.39	.89	4.91	21.36	73.73	78.64
	II	337	6.93	23.35	69	11.29	7.79	1100	15.59	17.15	-1.59	-6.81	33.36	73.45	66.64
	III	522	6.93	36.17	129	11.42	14.56	1100	21.35	25.82	-4.21	-11.64	40.25	71.39	59.75
K_2CrO_4	I	198	6.93	13.72	35	11.68	4.09	525	21.46	11.27	-1.64	-11.95	29.81	82.11	70.19
(500p.p.m.	II	286	6.93	19.82	54	11.13	6.01	450	34.62	15.58	-2.37	-8.93	30.32	78.61	69.68
in water)	III	449	6.93	31.12	85	10.30	9.46	600	33.16	19.90	1.76	5.65	30.40	63.95	69.60
$ZnCrO_4$	I	307	6.82	20.94	15.5	13.08	2.03	620	9.38	5.81	13.10	62.56	9.69	27.75	90.31
(1%) in	II	204	6.82	13.91	56.5	14.67	8.29	500	13.45	6.73	-1.11	-7.98	59.60	48.38	40.40
feed	III	264	6.82	18.00	70.0	14.72	10.30	500	11.91	5.96	1.74	19.66	57.22	23.11	42.78

Period		Grams Eaten	Per cent	Grams per Period	Grams of Feces	Per cent	Grams per Period	Milli-liters of Urine	Milli-grams per Milli-liter	Grams per Period	Grams Re-tained	Per cent Re-tained	Per cent in Feces	Per cent in Urine	Diges-tive Coeffi-cient
						CALCIUM BALANCE									
Control	I	262	.406	1.06	33	.820	.27	800	.62	.50	.29	27.36	25.47	47.17	74.53
	II	337	.406	1.37	69	.937	.65	1100	.73	.80	-.08	-5.84	47.45	58.39	52.55
	III	522	.406	2.12	129	.858	1.11	1100	.63	.69	.32	16.09	52.36	32.55	47.64
K_2CrO_4	I	198	.406	.804	35	.916	.32	525	.56	.29	.194	24.13	39.80	36.07	60.20
(500p.p.m.	II	286	.406	1.16	54	.895	.48	450	.37	.16	.52	44.83	41.38	13.79	58.62
in water)	III	449	.406	1.82	85	.729	.62	600	.55	.33	.84	47.80	34.07	18.13	65.93
$ZnCrO_4$	I	307	.449	1.378	15.5	1.032	.160	620	.467	.290	.928	67.35	11.61	21.04	88.39
(1%)	II	204	.449	.916	56.5	1.090	.616	500	.784	.392	-.092	-10.04	67.25	42.79	32.75
	III	264	.449	1.185	70.0	.972	.680	500	.400	.200	.305	35.74	57.38	16.88	42.62
						PHOSPHOROUS BALANCE									
Control	I	262	.456	1.19	33	.884	.292	800	.8324	.666	.132	19.49	24.54	55.97	75.46
	II	337	.456	1.54	69	.922	.684	1100	.7005	.771	.085	5.52	44.42	50.06	55.58
	III	522	.456	2.38	129	.915	1.180	1100	.8605	.947	.213	10.63	49.58	39.79	50.42
K_2CrO_4	I	198	.456	.90	35	.736	.258	525	1.5635	.821	-.179	-19.89	28.67	91.22	71.33
(500p.p.m.	II	286	.456	1.30	54	.835	.451	450	1.6355	.736	.113	8.69	34.69	56.62	65.31
	III	449	.456	2.05	85	.718	.610	600	1.7111	1.027	.313	20.14	29.76	50.10	70.23
$ZnCrO_4$	I	307	.499	1.532	15.5	1.262	.196	620	.312	.193	1.143	74.61	12.79	12.60	87.21
(1%)	II	204	.499	1.018	56.5	.887	.501	500	.206	.103	.414	40.67	49.21	10.12	50.79
	III	264	.499	1.317	70.0	.807	.565	500	.096	.048	.704	53.46	42.90	3.64	57.10

	Period	Grams Eaten	Per cent	Grams per Period	Amt. Feces	Per cent	Grams per Period	Grams Assimi-lated	Per cent Assimi-lated	Per cent in Feces	Digestive Coefficient
					FAT BALANCE						
Rabbit	I	262	3.21	8.41	33	1.15	.38	8.03	95.48	4.52	95.48
Control	II	337	3.21	10.82	69	1.29	.89	9.93	91.77	8.23	91.77
	III	522	3.21	16.76	129	1.92	2.48	14.28	85.20	14.80	85.20
K_2CrO_4	I	198	3.21	6.36	35	.94	.33	6.03	98.81	5.19	94.81
	II	286	3.21	9.18	54	1.37	.74	8.44	91.93	8.07	91.93
	III	449	3.21	14.41	85	1.81	1.54	12.87	89.31	10.69	89.31
$ZnCrO_4$	I	307	1.29	3.96	15.5	.86	.133	3.83	96.17	3.28	96.17
	II	204	1.29	2.63	56.5	1.19	.672	1.96	74.52	25.48	74.52
	III	264	1.29	3.41	70.0	1.16	.81	2.60	76.25	23.75	76.25
					CRUDE FIBER BALANCE						
Control	I	262	7.48	19.60	33	24.39	8.05	11.55	58.93	41.07	58.93
	II	337	7.48	25.21	69	30.04	20.73	4.48	17.78	82.22	17.78
	III	522	7.48	39.05	129	25.95	33.48	5.57	14.27	85.73	14.21
K_2CrO_4	I	198	7.48	14.81	35	30.19	10.57	4.24	28.63	71.37	28.63
	II	286	7.48	21.39	54	26.46	14.29	7.10	33.20	66.80	33.20
	III	449	7.48	33.59	85	26.25	22.31	11.28	33.59	66.41	33.59
$ZnCrO_4$	I	307	6.86	21.06	15.5	22.80	3.53	17.53	83.24	16.76	83.24
	II	204	6.86	13.99	56.5	24.14	13.64	.35	2.50	97.50	2.50
	III	264	6.86	18.11	70.0	24.35	17.04	1.07	5.91	94.09	5.91
					NITROGEN-FREE EXTRACT						
Control	I	262	54.69	143.3	33	40.85	13.48	129.8	90.58	9.42	90.58
	II	337	54.69	184.3	69	42.45	29.29	155.0	84.10	15.90	84.10
	III	522	54.69	285.5	129	43.75	56.44	229.1	80.25	19.75	80.25
K_2CrO_4	I	198	54.69	108.3	35	44.21	15.47	92.83	85.72	14.28	85.72
	II	286	54.69	156.4	54	46.04	24.86	131.54	84.10	15.90	84.10
	III	449	54.69	245.6	85	46.99	39.94	205.7	83.74	16.26	83.74
$ZnCrO_4$	I	307	54.93	168.6	15.5	48.46	7.51	161.09	95.55	4.45	95.55
	II	204	54.93	112.1	56.5	48.30	27.29	84.81	75.66	24.34	75.66
	III	264	54.93	145.0	70.0	39.84	27.89	117.11	80.77	19.23	80.77

BIOGRAPHY

William G. Gross was born at Franklin, Indiana, January 30, 1921. He received his grade school training in the same city and in May 1939 was graduated from Franklin High School. In September of the same year he matriculated at Franklin College, Franklin, Indiana and he received the Bachelor of Arts degree in May 1943.

In September 1943 he entered the Graduate School of Oklahoma A. and M. College where he has been employed as an analyst in Agricultural Chemistry Research until the present time.

Typed by Frances Stromberg