FLAME PHOTOMETRIC ANALYSIS OF SODIUM, POTASSIUM AND CALCIUM
IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS) FROM BIRTH
TO THE FOURTEENTH POSTNATAL WEEK

by

MARVIN FRANCIS HILL

A THESIS

submitted to

OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of

DOCTOR OF PHILOSOPHY

June 1955

APPROVED:

_____

Associate Professor of Zoology

In Charge of Major

_____

Chairman of the Department of Zoology

_____

Chairman of School Graduate Committee

_____

Dean of Graduate School

Date thesis is presented ___May 6, 1955___

Typed by Mary Adams

To My Parents

TABLE OF CONTENTS

FLAME PHOTOMETRIC ANALYSIS OF SODIUM, POTASSIUM AND CALCIUM IN THE GOLDEN HAMSTER (MESOCRICETUS AURATUS) FROM BIRTH TO THE FOURTEENTH POSTNATAL WEEK

INTRODUCTION

The chemical composition of mammals has interested many investigators. Among the first to make contributions in this field was von Bezold (5, pp.489-497)(6, pp.243-246), who was one of the first to make a chemical analysis of a human fetus, and who is the only worker found who has analyzed both humans and other species from a comparative standpoint.

Buckner (9, pp.7-8) studied the mineral content of the white rat during growth. His analysis, however, was made without the gastro-intestinal tract which was removed during carcass preparation.

Seventeen human fetuses of different ages were worked up for mineral content by Job and Swanson (15, pp.303-305). The first report found concerning the mineral content of an adult human was presented by Mitchell et al (20, pp.627-636). Contributions on the chemical development in utero, during growth, and composition in the adult was reported for rats, mice, rabbits, guinea pigs and man by Spray and Widdowson (27, pp.356-358)(28, pp.334-350)(30, pp.415-416)(31, p.627)(32, pp.208-212). The inorganic composition of ribs in the human fetus, premature, new born and adult was reported by Follis (12, p.224). Forbes and Caspar (13, pp.260-263) analyzed the mineral content of a human body and compared their data to Mitchell's.

Lilienthal (18, pp.503-506) presented evidence to show that the flame photometer was a reliable instrument for the determination

of the presence of metals in a substance by the measurement of the
intensity of their emitted spectral lines when excited in a flame.
This principle was used successfully by him in the spectro-chemical
analysis of many metallic elements. In this procedure, the sample
to be examined is placed in aequous solution and atomized under
controlled conditions, into an air-acetylene flame which is regulated
to provide uniform burning conditions. The light from the flame
enters the slit of a spectrograph, and spectrograms are prepared.
These spectrograms may be photometered in order to the intensity
of the spectral lines recorded. By calibration with solutions of
known composition and concentration, it is possible to correlate
the intensity of the spectral lines recorded. By calibration with
solutions of known composition and concentration, it is possible
to correlate the intensity of a given line with the amount of the
element present. The use of a high temperature acetylene flame
causes many of the metals contained in the sample to emit character-
istic radiations. This procedure, while both accurate and versatile
nevertheless requires both a complex apparatus and considerable
manipulation.

An instrument designed primarily for the analysis of sodium
and potassium in water samples was developed in the Stamford labor-
atories of the American Cyanamid Company and was described by Barnes
et al (3, pp.607-610). Because sodium and potassium were the metals
of primary interest, and because many metals emit their characteristic
spectral line when excited at the high temperature of the air-acetylene

flame, the lower temperatures of the air-propane flame were used in this instrument. This method of analysis, of measuring the emf generated in a photo cell by the spectral line of an element excited in a low temperature flame was called flame photometry.

Subsequently Berry et al (4, pp.20-23) developed a modification of this original Stamford instrument which involved a dual optical system. A uniform amount of a foreign metal was added routinely to all samples to be photometered. The light from the spectral line of the added foreign element passes through one optical system, while that from the spectral line of the element under consideration passes through the other. Measurements were made, not by absolute light intensities as in the earlier Stamford instrument but by intensity ratios. This modification was called the internal standard because of its similarity to the internal standard method used in spectral analysis. The element selected, due to technical considerations, was lithium. This choice may be considered objectionable in that it may occur as an impurity in mineral samples, but is fortunate for the biological worker due to the scarcity of lithium in biological materials.

Hald (14, pp.501-508) engaged this instrument in the determination of sodium and potassium in serum, whole blood, feces and in food. Comparisons between the data obtained by photometry and by chemical methods were done with a difference of ± 2.5 per cent to 3.5 per cent. It was further pointed out that photometry is much faster. With equally good results, Overman (21, pp.642-646) applied

the instrument to biological fluids.

Barlow (1, p.326) compared the photometric and chemical methods for the determination of cations in ashed tissue samples with an error of ±2.5 per cent.

Among others, the Perkin Elmer Company are building flame photometers. Their first series was the model 18, and the second, the model 52-A, was used in this study, the object of which was to follow the changing picture of sodium, potassium and calcium from birth to three weeks beyond sexual maturity in the golden hamster.

METHODS AND MATERIALS

The total of 270 hamsters used in this study were from the colony of Dr. H. H. Hillemann at Oregon State College. Their nutrition consisted of mixed whole grain, fruits, vegetables, salt and water. The cage litter was composed of dried, chopped alfalfa.

Time of copulation was noted as was the time of birth. Ten animals were taken for each day from birth through day 14 and ten for each week from the second through the fourteenth week. Ages were calculated from hour of birth for days one to 14, and from day of birth for weeks three through 14.

Following weaning, animals were placed in cages in pairs. It was felt that with only two animals per cage, both could get adequate nutrition. Since average figures were desired, an effort was made to mix litters. In order to eliminate the hazard of pregnancy, males and females were never placed together. Bogart et al (8, pp.363-368) state that pregnancy accelerates the growth rate of the white rat.

The animals were weighed on a triple beam balance, and then sacrificed by crushing some of their cervical vertebrae with a dull bone forceps but without breaking the skin. The abdominal cavity was then carefully opened, and the gastro-intestinal tract removed. The carcass was then placed in a pre-heated porcelain crucible of appropriate size. The gastro-intestinal tract, which had been slit longitudinally, sectioned and washed in distilled water, was added, and the sample dried to constant weight at 110° C. After drying,

the sample was placed in a cold muffle furnace, the temperature of which was raised gradually to 730°. The samples were held at this temperature until a white ash residue along with the teeth were all that was in evidence.

The ash residue and teeth were dissolved in concentrated, reagent grade nitric acid, a uniform amount of five cc. being added in each instance, to simplify making standard solutions.

The resulting solution was washed into volumetric flasks, and diluted to 100 cc. One cc. of this sample was later diluted to either 100 cc. or 200 cc., and this twice diluted sample was assayed by flame photometry.

Sodium, potassium and calcium concentrations were determined by the photo-electro measurement of the intensity of their character-istic emission spectra. These spectra were produced when dilute solutions of their salts were sprayed at a constant rate into a standard flame.

When an atom is heated, one of its electrons absorbs the added energy and jumps into an outer orbit where it revolves at a higher energy level. This electron is now in an unstable position. It tends to spring back to the orbit whence it came. As it returns to its original orbit it emits as light the difference in energy required for revolution in the two orbits concerned. The intensity and wave length of the light is related to the position and energy level of the moving electron. Hence, each element, when heated in a flame under standard conditions, emits a characteristic light

spectrum not given by any other element.

When energy is supplied at greatly different rates, e.g. at widely different temperatures, one element may produce a number of different emission spectra, according to the number of orbiting electrons available in its atoms to absorb the energy supplied. At very high temperatures, the outer electron may leave the field of the nucleus altogether, and an additional spectrum, or ion spectrum will then be produced. Thus, the higher the temperature, the more complex the spectra become.

In the propane-air flame, only the most easily excited spectral lines are produced, such as the first atomic spectra of the alkalies and alkaline earth metals. However, the flame must be sufficiently hot to vaporize the compounds to be analyzed. In the incandescent solid phase, continuous or band spectra are pro-duced, but these have small value for analytical purposes.

At high temperatures, interference phenomena occur between spectra of different elements. For example, with an oxy-acetylene flame at 3500° C., the presence of sodium increases the apparent intensity of the emission characteristic of potassium. This effect is minimal at the 1800° C. temperature of the propane-air flame.

An emission spectrum may be partially absorbed by atoms of another element, and well marked specific absorption will occur when unheated atoms of the same element are present. Both of these effects may be minimized by dilution. However, in flame photometry, the flame must supply energy at a sufficient rate to excite all of the atoms

delivered to it. Otherwise, the non-radiating atoms will absorb the light emitted by those that have been excited.

The main conditions for flame photometry are briefly as follows: An appropriately diluted solution of the material to be tested is delivered at a uniform rate to a flame of constant energy output. After vaporization, a variable number of atoms will be excited, and each atom will emit a constant amount of light of a characteristic wave length. This light is concentrated and selected by a suitable optical system, converted into electrical energy by a suitable photo cell and measured by a galvanometer.

In direct reading flame photometry, a single optical system and one photo-electric cell are used. The extent of the deflection of the galvanometer is a measure of the intensity of the emission spectra, and of the corresponding concentration of the elements in the heated specimen.

A flame supplying a constant amount of energy is obtained by using a modified Meeker burner with a fine mesh nickel grid. The flame is protected from cold air currents by a glass chimney. Narrow windows permit light from the central area of the flame to pass into the optical system while excluding light from the top and sides and base of the flame. This system of protection keeps the flame hot and steady but the rest of the instrument cool. This cooling is important because the output of selenium photo-electric cells varies with temperature changes.

The rate of atomization is governed by the air pressure. This

is delivered to the instrument through a constant pressure valve, at 10 lbs./sq. in.

The spray from the atomizer passes into a glass chamber designed to eliminate, through a trap, any coarse droplets which condense. The compressed air, now containing only very fine droplets, passes from the spray chamber through a short, wide tube, into a narrow metal preheating cylinder which surrounds the burner. It is essential that the spray enter the burner tangentially, and at a level above the air holes in the burner, lest the flame be blown out. The heat vaporizes the minute droplets of water and the compounds contained in solution. These are now in a gaseous phase so that characteristic emission spectra are produced.

In internal standard flame photometry, a constant proportion of lithium is added to all of the test and standard solutions. By the use of a double optical system and matched photo-electric cells the ratio of two different spectra are measured. These spectra are measured under conditions such, that any uncontrolled variation in the emission of one element is balanced by a similar alteration in the lithium internal element. The internal standard technique thus compensates for many of the serious errors which limit the value of direct reading flame photometry in the analysis of biological materials.

Lithium is a furtunate choice for the internal standard element because it seldom occurs in any significant quantity in biological materials. It gives an intense line spectrum midway

between the spectra of sodium and potassium. With a constant proportion of lithium added to all of the standard solutions and to the unknowns to be tested, the potential produced by the lithium light falling on one photo cell is opposed by a potential produced by light from the unknown element falling upon the opposite photo cell. These potentials are balanced by a potentiometer, having a dial calibrated from zero to 100. A calibration curve is constructed by plotting the differing potentiometer readings against the concentrations of the standard solutions. A potentiometer reading is the reading on the potentiometer dial, when the galvanometer of the instrument is brought to a null point.

The instrument chosen for this study was a Perkin Elmer model 52-A, and the internal standard method was used throughout. Lithium nitrate C.P. was made up at 1,000 ppm. for the internal standard, and five cc. of this solution were added to each of the solutions, both standard and unknown.

The method was tested in the following manner. Twenty samples each of heart, liver, and hamburger were fortified with sodium, potassium and calcium at different levels. These samples were carried through the preparation previously described and then assayed on the photometer. The average error for sodium was ±2.6 per cent, for potassium ±2.3 per cent and for calcium ±3.4 per cent.

Lithium has an emission spectral line at 6708 Å, sodium at 5980 Å, and potassium at 7665 Å. Calcium exhibits several band spectra in this range.

Calibration curves of widely separate concentrations were used first, to locate the concentration of the unknown. Following this, a new series of known dilutions whose concentrations more closely approximated the unknown were made. Over a narrow range of 20 to 25 ppm, four to five different concentrations were used.

The highest concentration was potentiometrically balanced in the instrument at a scale reading of 100. Distilled water was used as zero, with the remaining concentrations in between. In this manner, a narrow range of concentrations could be spread over a full scale deflection, and the unknown located between two adjacent points.

As the animal ash was taken up in five cc. of concentrated nitric acid, five cc. of this substance was also added to each of the standard solutions.

DISCUSSION

The data obtained during this investigation are presented in Table I and form the basis of the graphs (Figs. 1,2,3,4,5,6,7).

The average total wet weights are plotted against age in Figure 1. From birth to the end of the first day, there is no increase in wet weight, while between days one and two there is an increase of 26 per cent, and a further increment of 5 per cent between the third and fourth days. Between birth and the end of the second week, there is an increase of 560 per cent. The increase of 426 per cent in the 12 weeks from week two to week 14, is 134 per cent less than the increase in the first two weeks. Buckner and Peter (9, pp.7-8) report a 498 per cent increase in the wet weight of the white rat in the two to 14 week period. For the first 15 days of postnatal life, increases of 500 per cent in the mouse and 39 per cent in the guinea pig are reported (27, p.356). The increase in the hamster of 560 per cent in the first 14 days, is 60 per cent greater than the increase in the mouse for a similar period.

Among the scattered data points, it is possible to construct a curve which is regular. The erratic points might be smoothed out by a larger sampling. Since the animals for the wet weight data were weighed alive, food in some stomachs may have introduced an error.

Figure 2 plots total dry weights against time. The increase proceeds at a greater rate than that of the wet weights.

From birth to the end of day one, there is an increase of seven per cent and between days one and two, an increase of 25 per cent. Compared to an increase in wet weight of five per cent from days three to four, we have a 34 per cent increase of dry weight. In the time interval between birth and the end of week two, an increase in dry weight of 832 per cent is found; this is 272 per cent greater than the wet weight increase for the same period. An increase in dry weight of 8,388 per cent, which is over 5000 per cent more than the wet weight per cent increase for the same period is evidenced in the 14 weeks of the investigation.

There is 10 per cent dry matter in the hamster at birth, compared with 10 per cent in the 8.4 month human fetus (15, p.304).

Figure 3 is a plot of the average ash weights against time. From birth to day one, there is an increase of 8 per cent in the average ash followed by a 10 per cent increase from days one to two, and from days three to four, an 18 per cent increase. An increase of 59 per cent from days 12 to 13 may be noted.

During the first 14 days, the ash residue increases 469 per cent faster than during the next 12 weeks. The per cent increase during the first two weeks is 908 per cent and during the interval between weeks two and four, 439 per cent. An overall increase of 5,340 per cent from birth to the end of the 14th week may be noted.

Buckner (9, pp.6-8), in rats, found a 757 per cent increase in the crude ash from weeks two to 14. He found a 1.79 per cent ash at day 14, which is here compared with 2.30 per cent found in

the hamster, and a 2.56 per cent ash at week 14, compared with the hamster's 2.35 per cent.

From studies upon the adult human body, Forbes and Casper (13, p.361) report an ash content of 5.43 per cent, Mitchell (20, p.628) 4.84 per cent and Widdowson (30, p.115) 6.0 per cent.

A constant increase in calcium over the period studied is shown by the graph (Fig. 4). From birth until the ninth day, its concentration increases by 165.3 per cent, and by 600 per cent from days nine to 14. In the last 12 weeks studied, from weeks 2 to 14, there is an increase of 1,051 per cent, with an overall increase from birth to the end of week 14 of 21,308 per cent.

Because of its relation to the growth of the skeleton, this increase in calcium during the period of development is not surprising. A study of this problem beyond the age of 14 weeks would be of interest.

The calcium concentration per wet weight was found to be 0.15 per cent in the hamster at birth, 0.30 per cent in the rat and 0.34 per cent in the mouse (31, p.627). Von Bezold (6, p.250) reports a calcium per wet weight ratio of 0.32 per cent and 0.31 per cent in the new-born mouse and bat respectively.

In the 14-week hamster, there was 0.95 per cent calcium. Mitchell (20, p.628) reports 1.59 per cent, Forbes and Casper (13, p.361) 1.9 per cent, and Widdowson (30, p.115) 2.0 per cent; all figures pertain to the adult human body. Buckner (9, pp.6-8) working on rats, reports 0.99 per cent calcium per wet weight in

the 14-week rat; it increases to 1.47 per cent at 40 weeks.

These figures indicate that with respect to calcium accumulation, the hamster presents a physiological immaturity at the age of 14 weeks.

The overall picture of sodium (Fig. 5) is one of increase. Day nine and week three may have significance, since Job (15, p.304) also presents a picture of erratic increase in sodium in the human fetus.

An increase in the overall concentration of sodium is not surprising, since it is a constituent of both the blood plasma and extracellular fluid. Save for a marked increase of 87 per cent between days four and six, its concentration increases gradually.

In the period from birth until the end of the second week, an increase of 660 per cent for sodium may be observed. This increase is 1,099 per cent less than that for calcium, but 407 per cent more than that for potassium over the same period.

Sodium increased 219 per cent in the interval between the second and fourteenth weeks, which is 441 per cent less than the increase in the first 14 postnatal days. From birth to the end of week 14, sodium increased by 2,326 per cent.

Potassium (Fig. 6) like sodium, shows a decrease in concentration between weeks 13 and 14. The sodium decrease is only 2.6 per cent while that of potassium is 21.4 per cent. From birth to day five, the potassium content increases 60 per cent and then levels off for the next five days. From day 12 to day 14, there is

an increase of 86.6 per cent. This rate of increase levels off some-
what to week 11, when there is a 57.2 per cent increase to week 13.

From birth to the end of the second week, potassium increases
253 per cent, and from the second to the 14th week, 322 per cent. A
total increase of 1,393 per cent from birth to the end of week 14 may
be noted.

Buckner (9, pp.6-8) reports 0.04 per cent potassium in the
four-week rat carcass compared with 0.15 per cent found in the
hamster. In the 14-week rat carcass, he reported 0.03 per cent,
compared with 0.14 per cent in the hamster.

Figure 7 is the sum of the average sodium, potassium and
calcium plotted against time. The curve is influenced by calcium,
which is the dominant cation considered. The daily sum of the
cations considered is approximately one-half of the total "daily"
ash. Other components of the ash must include iron, copper and
zinc, among others.

In a study of this type there are many sources of possible
error. To prevent contamination by sodium leeched from container
walls, Pyrex should be used for all solutions. New glassware should
be soaked and washed for the same reason. The rotary grinding motion
of seating glass stoppers should not be used, as sodium from the
glass ground off will contaminate the solution.

Environmental sources of contamination are also important.
Dust in the air or the handling of soap powder in the instrument
room will cause a large error in sodium or potassium. Potassium

in quantity is also contributed by tobacco smoke. Analytical procedures regarding glassware should be observed. An ever present source of error may be the sodium present in fingerprints.

The top of the muffle furnace should be inspected to eliminate any possible infall of particles into the samples.

Air under constant pressure must be cleaned by passing through a filter before entering the photometer. Gas pressure must also be constant since too low a pressure produces a flame with objectionable incandescant streaks. When using filter paper for ashing, Whatman number 40 is recommended.

Extensive surveys of the sources of error in flame photometry may be found in Parks (22, pp.823-824), Sommer (26, pp.277-281), Bells (8, pp.1078-1079) and Elliott (10, pp.111,121).

SUMMARY

A total of 270 hamsters were studied for wet, dry and ash weights, as well as for total sodium, potassium and calcium, by the use of a Perkin Elmer Flame Photometer (Model 52-A) involving an internal standard.

Ten animals were studied for each day from birth to the second week, and ten animals for each week from the second through the fourteenth week.

The range of average wet weights varied from 2.3 grams per animal at birth to 80 grams at the end of week fourteen. Average dry weights increased from 0.25 gram at birth to 21.22 grams at week fourteen, and average ash weights ranged from 0.034 gram at birth to 1.888 grams at the fourteenth week. The sum of the average sodium, potassium and calcium increased from 14.47 milligrams at birth to 955 milligrams at week fourteen.

The average calcium content of the animals varied from 3.55 milligrams at birth to 760 milligrams at the fourteenth week; sodium ranged from 3.42 milligrams at birth to 83 milligrams at week fourteen; and potassium increased from 7.5 milligrams at birth to 112 milligrams at the fourteenth week.

From birth to the end of week fourteen wet weight increased 3,378 per cent, dry weight 8,388 per cent, ash weight 5,340 per cent, sodium 2,326 per cent, potassium 1,393 per cent and calcium 21,308 per cent.

In the wet carcass, the percentage of calcium increases from 0.15 per cent at birth to 0.43 per cent at the end of week two, and 0.95 per cent at the end of week 14.

In the new-born, there is 0.14 per cent sodium in the wet carcass, and 0.17 per cent at week two, an increase of 0.03 per cent. By week 14, the percent of sodium has decreased to 0.10 per cent, a loss of 0.07 per cent.

The percentage of potassium in the wet carcass of the new-born is 0.32 per cent. By the end of week two, it has dropped to 0.17 per cent, and decreases further to 0.14 per cent by week fourteen.

TABLE I.

	Average Weight in Grams			Average Weight in Milligrams			
	Wet	Dry	Ash	Ca	Na	K	Cation
Newborn	2.3	0.2509	0.0347	3.55	3.42	7.50	14.47
Day 1	2.3	0.2695	0.0378	4.31	4.41	7.81	16.53
" 2	2.9	0.3377	0.0417	4.60	6.30	8.32	19.22
" 3	3.8	0.4070	0.0460	5.13	7.52	9.10	21.93
" 4	4.0	0.5481	0.0543	5.40	8.20	9.81	23.41
" 5	4.4	0.7714	0.0786	5.70	13.0	12.0	30.10
" 6	5.97	0.9125	0.1132	6.19	15.0	11.0	32.29
" 7	4.38	1.2702	0.1325	6.80	15.4	11.2	33.40
" 8	8.8	1.6306	0.1526	8.30	16.5	12.0	36.80
" 9	9.4	1.6503	0.1530	9.42	15.2	12.2	36.82
" 10	8.5	1.6705	0.1536	20.0	16.5	12.0	48.50
" 11	12.9	1.6898	0.1543	24.0	17.0	13.5	54.50
" 12	9.0	1.7103	0.1563	35.0	17.7	14.2	66.90
" 13	15.7	1.7973	0.2489	54.0	20.2	18.1	92.30
Week 2	15.2	2.3300	0.3500	66.0	26.0	26.5	118.5
" 3	15.3	3.7900	0.4331	94.0	23.5	30.0	147.5
" 4	24.1	5.2424	0.6659	160.0	32.5	37.0	229.5
" 5	52.0	7.3132	0.7937	182.0	34.5	38.1	254.6
" 6	35.0	9.3761	0.9220	265.0	40.2	44.0	349.2
" 7	51.0	13.5259	1.1892	273.0	43.2	51.5	367.7
" 8	66.4	15.4391	1.2389	341.0	47.0	58.3	446.3
" 9	65.0	17.3546	1.2980	433.0	60.5	67.2	560.7
" 10	77.0	17.9677	1.4430	486.0	65.5	75.0	626.5
" 11	77.0	18.5802	1.6260	524.0	71.5	86.5	682.0
" 12	73.0	19.8100	1.6600	580.0	74.8	107.5	762.3
" 13	80.0	20.5152	1.7734	653.0	85.2	136.0	874.2
" 14	80.0	21.2213	1.8880	760.0	83.0	112.0	955.0

TABLE I. (Continued)

		Water Content	Percent Water Wet Weight	Percent Ash		K/Na
				Wet Weight	Dry Weight	
New Born		2.0491	88.6	1.50	13.83	2.19
Day	1	2.0305	88.2	1.64	14.02	1.78
"	2	2.5623	88.2	1.43	12.34	1.32
"	3	3.3930	89.2	1.21	11.30	1.21
"	4	3.4519	86.2	1.35	9.90	1.19
"	5	3.6286	82.2	1.78	11.05	0.92
"	6	5.0575	84.5	1.89	12.40	0.73
"	7	3.1098	70.7	3.01	10.43	0.72
"	8	7.1694	81.3	1.73	9.35	0.72
"	9	7.7497	82.3	1.62	9.27	0.80
"	10	6.8295	80.2	1.80	9.19	0.73
"	11	11.2102	86.8	1.19	9.13	0.79
"	12	7.2897	80.8	1.73	9.13	0.80
"	13	13.9027	88.5	1.58	13.84	0.89
Week	2	12.870	84.6	2.30	15.20	1.01
"	3	11.410	74.5	2.83	11.58	1.27
"	4	18.8576	78.2	2.76	12.70	1.13
"	5	44.6868	85.9	1.52	10.85	1.10
"	6	25.623	73.2	2.63	9.83	1.09
"	7	37.474	73.4	2.33	8.79	1.19
"	8	50.960	76.7	1.86	8.02	1.24
"	9	47.645	73.2	1.95	7.47	1.11
"	10	59.032	76.6	1.87	8.03	1.14
"	11	58.419	75.8	2.11	8.75	1.20
"	12	53.190	72.8	2.27	8.37	1.43
"	13	59.484	74.3	2.21	8.64	1.59
"	14	58.778	73.4	2.36	8.89	1.35

TABLE I. (Continued)

		Increase in Grams		Increase in Milligrams				
		Wet Weight	Dry Weight	Ash Weight	Na	K	Ca	Cation
New Born		-	-	-	-	-	-	-
Day	1	0.00	0.0186	3.10	0.99	0.31	0.76	2.06
"	2	0.60	0.0682	3.90	1.89	0.51	0.29	2.69
"	3	0.90	0.0693	4.30	1.22	0.78	0.53	2.71
"	4	0.20	0.1411	8.30	0.68	0.71	0.27	1.48
"	5	0.40	0.2233	24.30	4.80	2.19	0.30	6.69
"	6	1.57	0.1411	34.60	2.00	-1.1	0.49	2.19
"	7	-1.59	0.3577	19.30	0.40	0.2	0.61	1.11
"	8	4.42	0.3604	20.10	1.10	0.8	1.50	3.40
"	9	0.60	0.0197	0.40	-1.30	0.2	1.12	0.02
"	10	-0.90	0.0202	0.60	1.30	-0.2	10.58	11.68
"	11	4.40	0.0193	0.70	0.50	1.5	4.00	6.00
"	12	-3.90	0.0205	2.00	0.70	0.7	11.00	12.48
"	13	6.70	0.0870	92.60	2.50	3.9	19.00	25.40
Week	2	-0.50	0.5327	101.1	5.8	8.4	12.0	26.2
"	3	0.1	1.4600	83.1	-2.5	3.5	28.0	29.0
"	4	8.80	1.4524	232.8	9.0	7.0	66.0	82.0
"	5	27.90	2.0708	127.8	2.0	1.1	22.0	25.1
"	6	-17.00	2.0629	128.3	5.1	5.9	83.0	94.6
"	7	16.00	4.1498	267.2	3.0	7.5	8.0	18.5
"	8	15.40	1.9132	49.7	3.8	6.8	68.0	78.6
"	9	-1.40	1.9155	59.1	13.5	8.9	92.0	144.4
"	10	12.00	0.6131	145.0	5.0	7.8	53.0	65.8
"	11	0.00	0.6125	183.0	6.0	11.5	38.0	55.5
"	12	3.00	1.2298	34.0	3.3	21.0	56.0	80.3
"	13	7.00	0.7052	113.4	10.4	28.5	73.0	111.9
"	14	-3.83	0.7061	114.6	-2.2	-24.0	7.0	80.8

TABLE I. (Continued)

PERCENT CALCIUM

		Wet Weight	Dry Weight	Ash Weight	Cation Weight
New Born		0.15	1.41	10.23	24.03
Day	1	0.18	1.59	11.40	24.98
"	2	0.15	1.36	11.03	23.93
"	3	0.13	1.26	11.15	23.39
"	4	0.13	0.98	9.94	23.06
"	5	0.12	0.73	7.25	18.93
"	6	0.10	0.67	5.46	19.17
"	7	0.15	0.53	5.13	19.76
"	8	0.09	0.50	5.43	22.55
"	9	0.10	0.57	6.14	25.58
"	10	0.23	1.19	13.02	41.23
"	11	0.18	1.42	15.55	44.03
"	12	0.38	2.04	22.39	52.31
"	13	0.34	3.00	21.69	58.50
Week	2	0.43	2.83	18.85	55.69
"	3	0.61	2.48	21.70	63.72
"	4	1.25	3.05	24.02	69.71
"	5	0.35	2.48	22.93	71.48
"	6	0.75	2.82	28.74	75.88
"	7	0.53	2.01	22.95	74.24
"	8	0.51	2.20	27.52	76.40
"	9	0.66	2.49	33.35	77.22
"	10	0.63	2.70	33.67	77.57
"	11	0.68	2.82	32.22	76.83
"	12	0.79	2.92	34.93	76.08
"	13	0.81	3.18	36.82	74.69
"	14	0.95	3.58	40.25	79.58

TABLE I. (Continued)

P E R C E N T   S O D I U M

	Wet Weight	Dry Weight	Ash Weight	Cation Weight
New Born	0.148	1.36	9.85	23.63
Day 1	0.191	1.59	11.66	26.67
" 2	0.217	1.86	15.10	32.77
" 3	0.197	1.84	16.34	34.29
" 4	0.205	1.49	15.10	35.02
" 5	0.295	1.68	16.53	43.18
" 6	0.251	1.64	13.25	46.45
" 7	0.351	1.21	11.62	46.10
" 8	0.187	1.01	10.81	44.83
" 9	0.161	0.92	9.93	41.28
" 10	0.194	0.98	10.74	34.02
" 11	0.131	1.00	11.01	31.19
" 12	0.196	1.03	11.32	26.45
" 13	0.128	1.12	8.11	21.88
Week 2	0.171	1.11	7.42	21.94
" 3	0.153	0.62	5.42	15.93
" 4	0.134	0.61	4.88	14.16
" 5	0.066	0.47	4.32	13.55
" 6	0.114	0.42	4.36	11.51
" 7	0.084	0.31	3.63	11.75
" 8	0.070	0.30	3.79	10.53
" 9	0.093	0.34	4.66	10.79
" 10	0.085	0.36	4.53	10.45
" 11	0.092	0.38	4.39	10.48
" 12	0.102	0.37	4.50	9.81
" 13	0.106	0.41	4.80	9.74
" 14	0.103	0.39	4.39	8.69

TABLE I. (Continued)

PERCENT POTASSIUM

|		Wet Weight	Dry Weight	Ash Weight	Cation Weight
New Born		0.3260	2.98	21.61	51.83
Day	1	0.3395	2.89	20.66	47.24
"	2	0.2868	2.46	19.95	43.28
"	3	0.2394	2.23	19.78	41.49
"	4	0.2295	1.78	18.06	41.90
"	5	0.2727	1.55	15.26	39.86
"	6	0.1842	1.20	9.71	34.06
"	7	0.2557	0.88	8.45	33.53
"	8	0.1363	0.73	7.86	32.60
"	9	0.1297	0.73	7.97	33.13
"	10	0.1411	0.71	7.81	24.74
"	11	0.1046	0.79	8.74	24.77
"	12	0.1577	0.83	9.08	21.22
"	13	0.1152	1.00	7.27	19.60
Week	2	0.1743	1.13	0.79	22.36
"	3	0.1960	0.79	6.92	20.33
"	4	0.1535	0.70	5.55	16.12
"	5	0.0732	0.52	4.77	14.96
"	6	0.1257	0.46	4.77	12.60
"	7	0.1009	0.38	4.33	14.00
"	8	0.0878	0.37	4.70	13.06
"	9	0.1033	0.38	5.17	11.98
"	10	0.0974	0.41	5.19	11.97
"	11	0.1123	0.46	5.31	12.68
"	12	0.1472	0.54	6.47	14.10
"	13	0.1700	0.66	7.66	15.55
"	14	0.1400	0.52	5.93	11.72
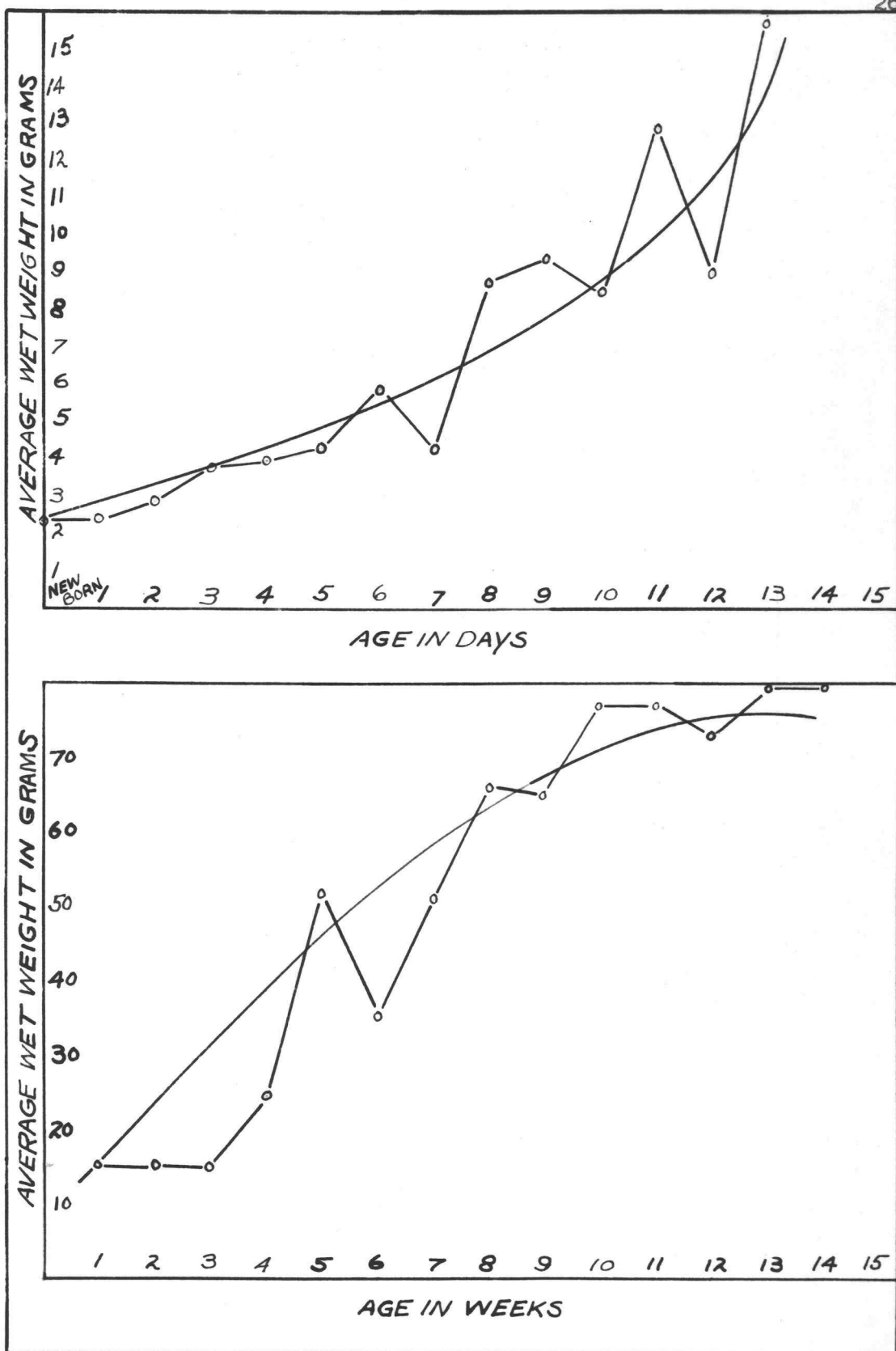
Figure 1.
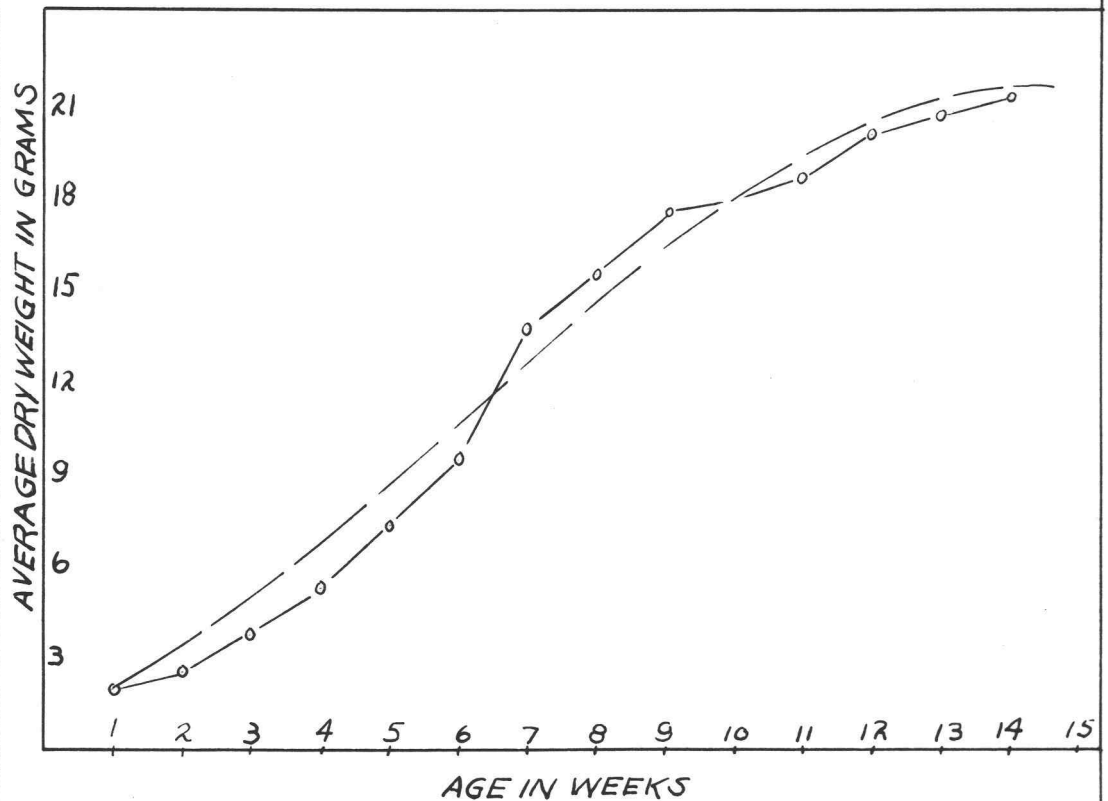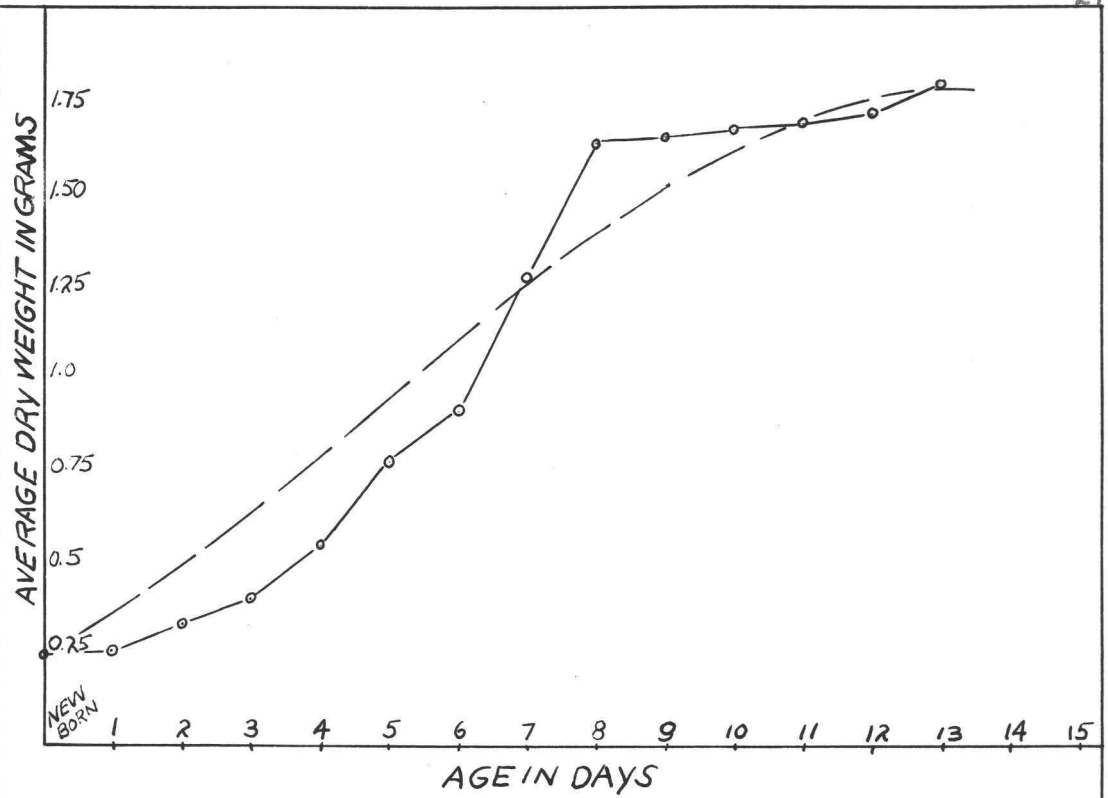
Figure 2.
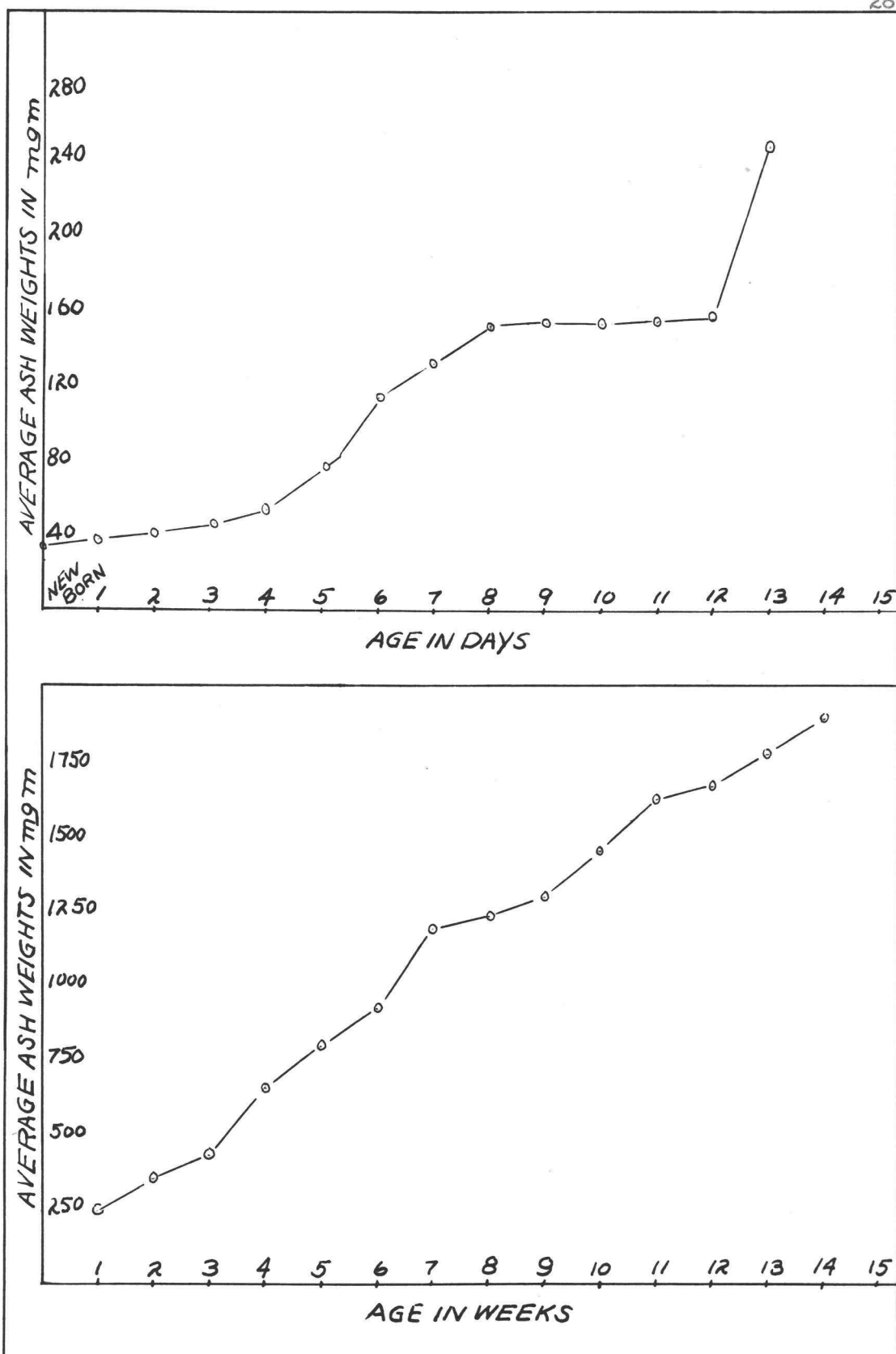
Figure 3.
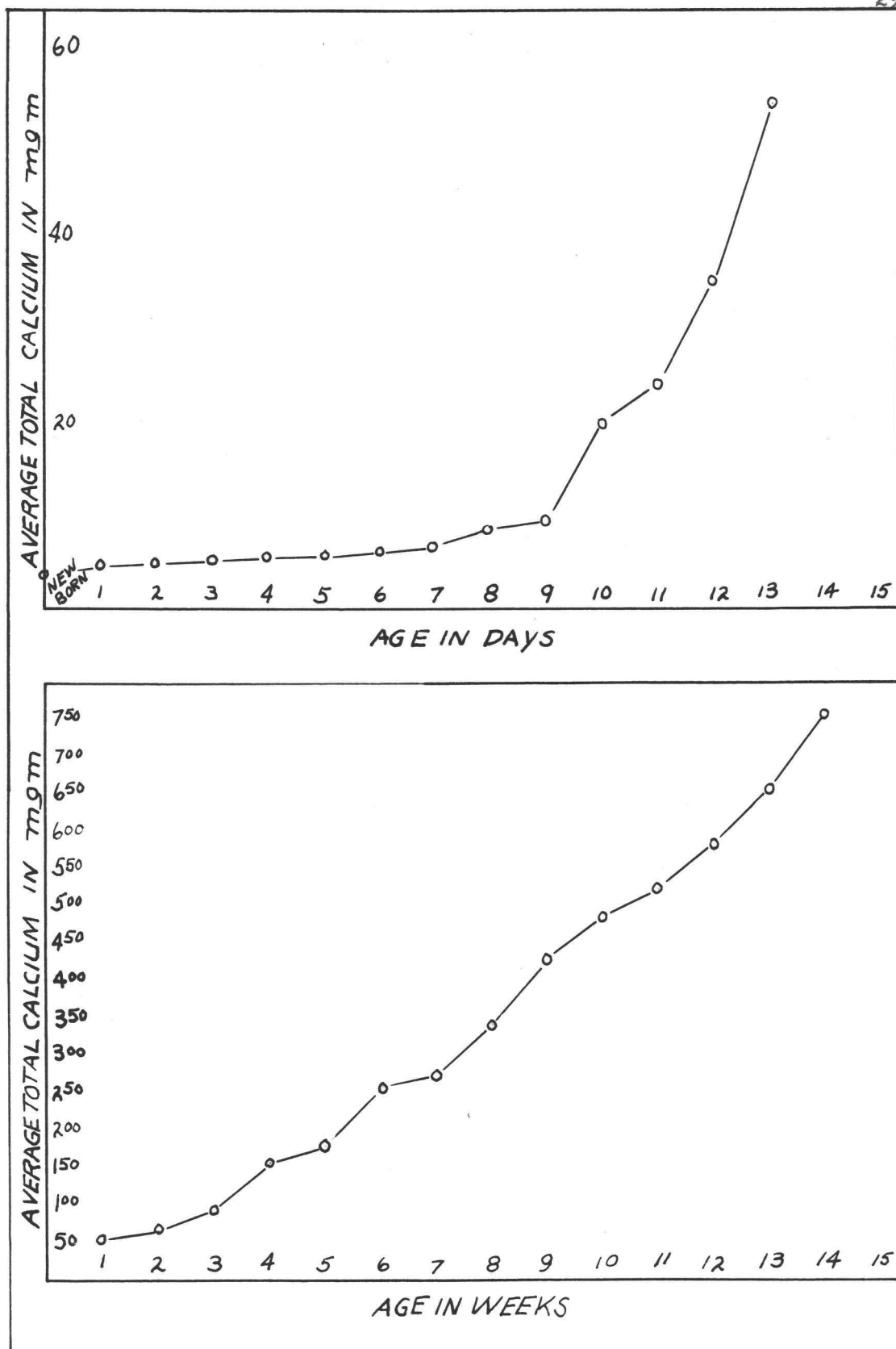
Figure 4.
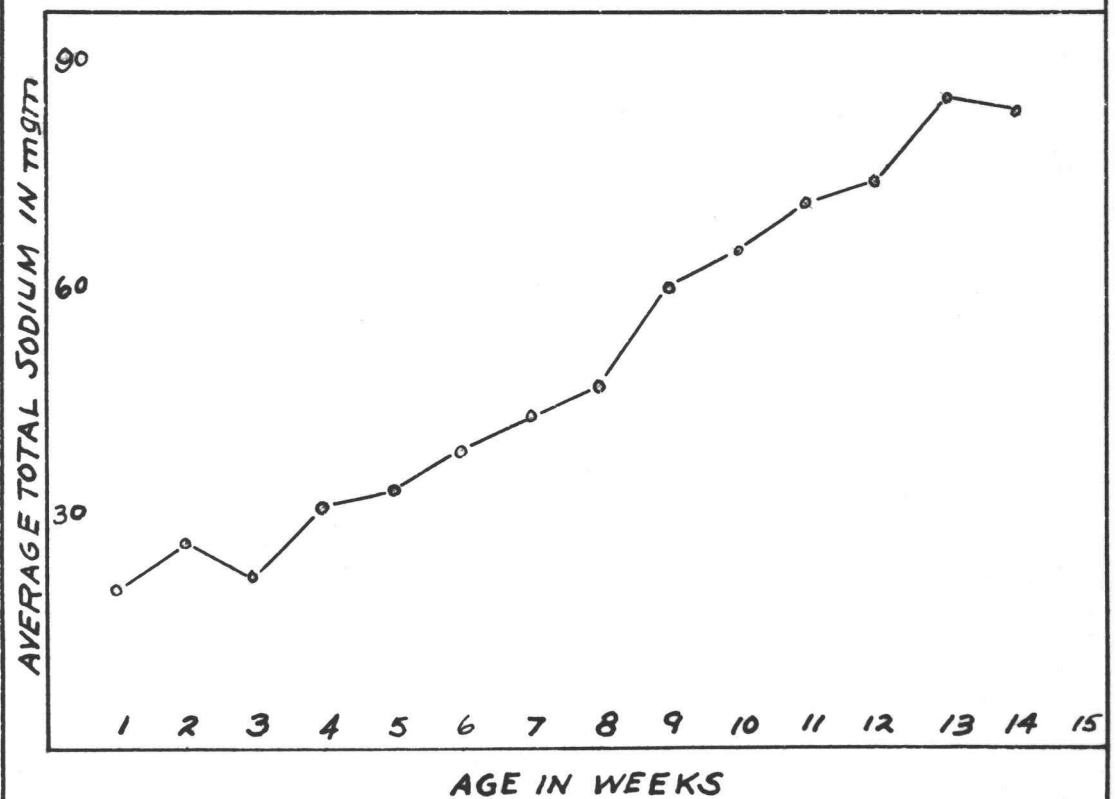
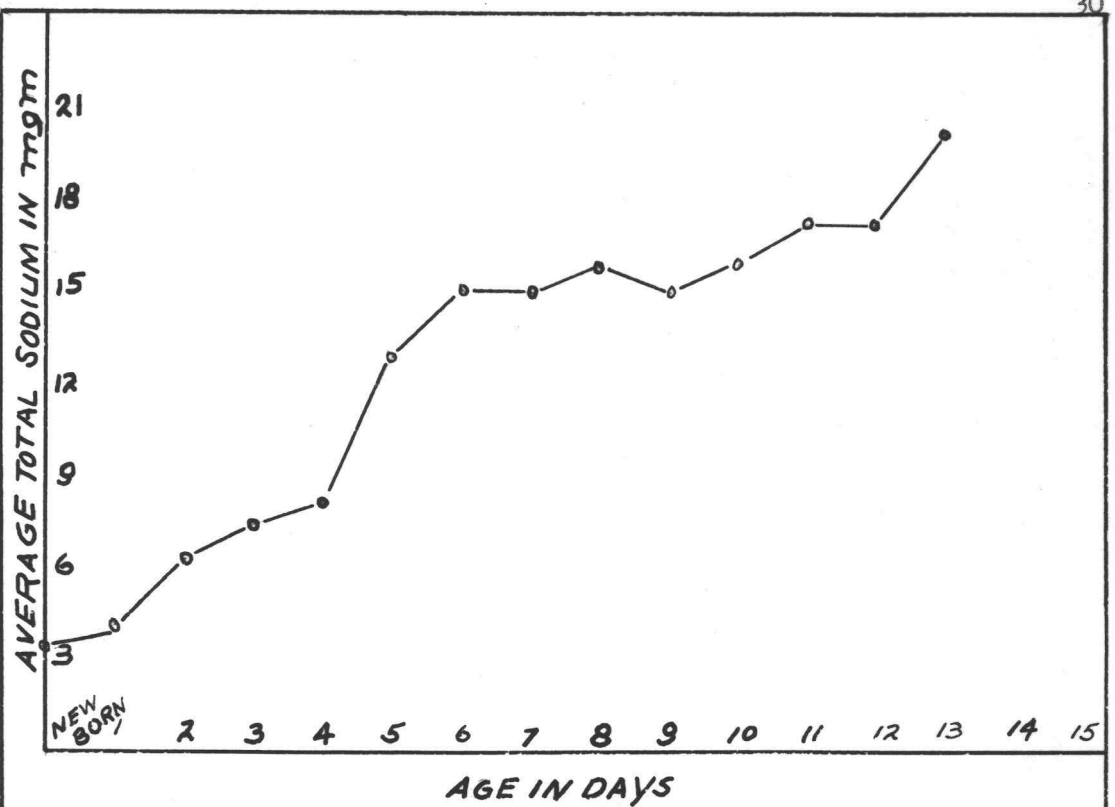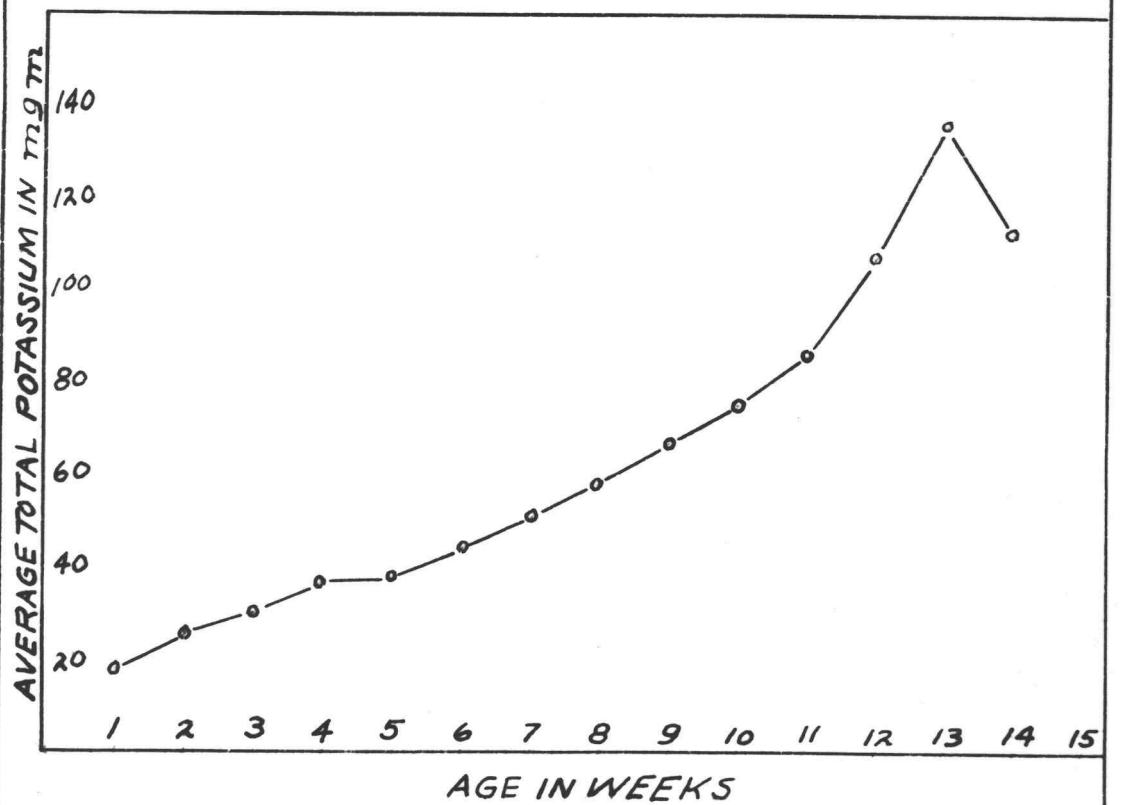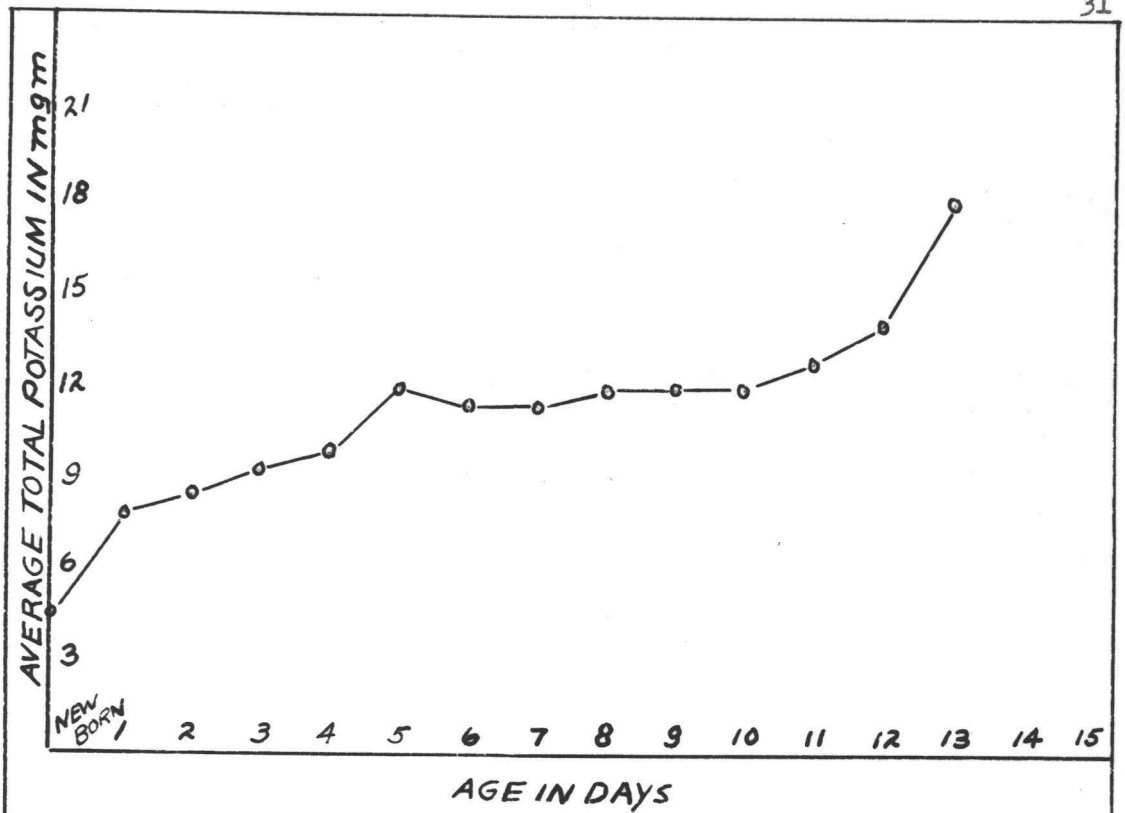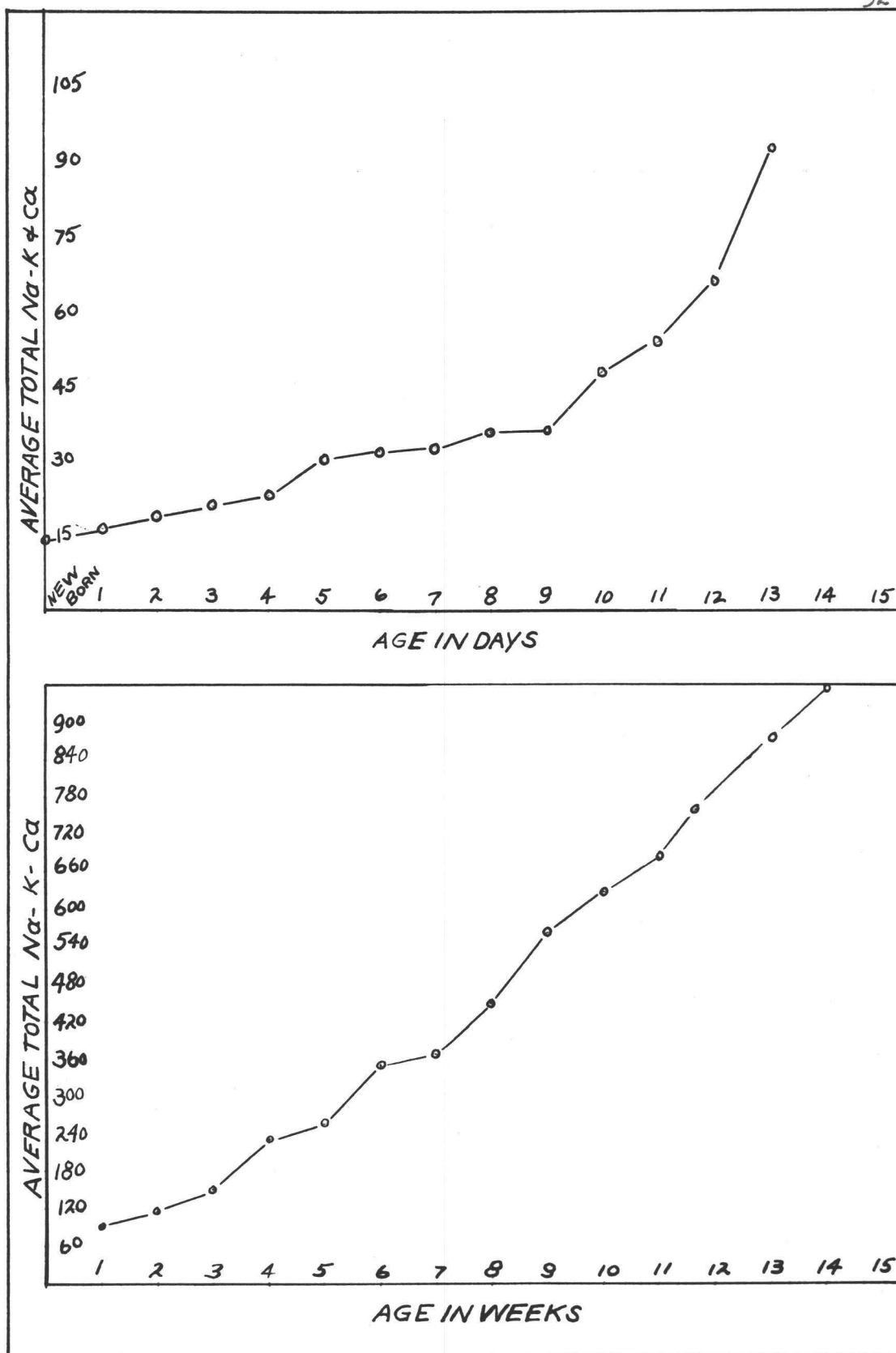Figure 5.

Figure 6.

Figure 7.

BIBLIOGRAPHY

1. Barlow, J. S. and J. F. Manery. A comparison of the method of
        flame photometry with the chemical method for the determi-
        nation of cations in tissues. Canadian journal of medical
        science 31:326-37. 1953.

2. Barnes, Bowling R., J. W. Berry and W. B. Hill. The flame
        photometer. Engineering and mining journal 149:92-94.
        1948.

3. Barnes, Bowling R. et al. Flame photometry, a rapid analytical
        procedure. Industrial engineering chemistry. Analytical
        edition 17:605-611. 1945.

4. Berry, J. W. et al. Improved method of flame photometry.
        Industrial engineering chemistry. Analytical edition
        18:19-24. 1946.

5. Bezold, Albert v., Untersuchungen über die Vertheilung von
        Wasser, organischer Materie und anorganischen Verbindungen
        in Thierreiche. Zeitschrift für wissenschaftliche
        Zoologie 8:487-503. 1857.

6. Bezold, Albert v., Das chemische Skelett der Wirbelthiere.
        Ein physiolog-chemischer Versuch. Zeitschrfit für wissen-
        schaftliche Zoologie 9:240-257. 1858.

7. Bills, Charles E., et al. Reduction of error in flame photo-
        metry. Analytical chemistry 21:1076-1080. 1949.

8. Bogart, R., et al. The influence of reproductive condition
        upon growth in the female rat. American journal of
        physiology 128:355-371. 1939.

9. Buckner, G. D. and A. M. Peter. The mineral content of the
        normal white rat during growth. Journal of biological
        chemistry 54:5-9. 1922.

10. Eliott, Francis H. Determination of the characteristics of a
        flame photometer and effects of interfering substances.
        Canadian journal of technology 29:111-122. 1951.

11. Flexner, Louis B. Changes in the chemistry and nature of the
        cerebrospinal fluid during fetal life in the pig.
        American journal of physiology 124:131-135. 1938.

12. Follis, Richard H. The inorganic composition of the human rib.
    Journal of biological chemistry 194:223-226. 1951.

13. Forbes, R. M. and A. R. Casper. The composition of the adult
    human body as determined by chemical analysis. Journal
    of biological chemistry 203:359-366. 1953.

14. Hald, Pauline M. The flame photometer for the measurement of
    sodium and potassium in biological fluids. Journal of
    biological chemistry 167:499-510. 1947.

15. Job, Vivian, and William W. Swanson. Mineral growth of the
    human fetus. American journal of the diseases of child-
    ren 47:302-306. 1934.

16. Janke, Bodo and Dorothea Scharpff. Klinische untersuchungen
    über den Natrium, Kalium und Kalzium Stoffwechsel.
    Deutsche medizinische Wochenschrift 78:786-788. 1953.

17. Keirs, R. J. and S. J. Speck. Determination of milk minerals
    by flame photometry. Journal of dairy science 33:413-423.
    1950.

18. Lilienthal, J. L. et al. A reference base and system of
    analysis of muscle constituents. Journal of biological
    chemistry 182:501-508. 1950.

19. Mazzamaro, Patrick and G. Tatoian. Determination of small
    concentrations of sodium. Analytical chemistry 26:1512-
    1513. 1954.

20. Mitchell, H. H., et al. The chemical composition of the adult
    human body and its bearing on the biochemistry of growth.
    Journal of biological chemistry 158:625-637. 1945.

21. Overman, Richard R., and A. K. Davis. The application of
    flame photometry to sodium and potassium determinations
    in biological fluids. Journal of biological chemistry
    168:641-649. 1947.

22. Parks, Thomas, H. O. Johnson and L. Lykken. Errors in the use
    of the Perkin Elmer flame photometer, model 18, for the
    determination of the alkali metals. Analytical chemistry
    20:822-825. 1948.

23. Pro, Maynard J. and Alex P. Mathers. Metallic elements in
    wine by flame photometry. Journal of the association of
    official agricultural chemists 37:945-960. 1954.

24. Ramsey, J. A. Determination of sodium in small volumes of
       fluid by flame photometry. Journal of experimental
       biology 27:407-419. 1950.

25. Seger, Alma et al. Flame photometry. American journal of
       medical technology 18:281-289. 1952.

26. Sommer, Anna J. Uses and abuses of the flame photometer.
       American journal of medical technology 17:276-282. 1951.

27. Spray, Christine M. A study of some aspects of reproduction by
       means of chemical analysis. British journal of nutrition
       4:354-360. 1950.

28. Spray, Christine M., and E. M. Widdowson. The effect of growth
       and development on the composition of mammals. British
       journal of nutrition 4:332-353. 1950.

29. Stanford, George and Leah English. Use of the flame photometer
       in rapid soil tests for potassium and calcium. Agronomy
       journal 41:446-447. 1949.

30. Widdowson, E. M., R. A. McCause and C. M. Spray. Concerning the
       chemical composition of the human body. Clinical science
       10:113-117. 1951.

31. Widdowson, E. M. Chemical constitution of newly born mammals.
       Nature 166:626-628. 1950.

32. Widdowson, E. M. and C. M. Spray. Chemical development in
       utero. Archives of the diseases of childhood 26:205-214.
       1951.