THE ANTIRACHITIC POTENCY OF ARIZONA SUNSHINE

by

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INTRODUCTION

The ultimate goal in the study of any disease is to be able to recognize and describe its cause, explain the resulting disturbances and effect a cure or preventive measure or both. In the case of the disease known as rickets the etiology is definitely established and successful curative and preventive measures exist. Rickets is a disease in which mineral metabolism is disturbed in such a way that defective bone development results. The prevention and cure of rickets in view of investigations to date depend upon one of three factors: (1) the mineral content of the diet, (2) the vitamin D content of the diet or (3) irradiation with ultraviolet light. It has been proved that through proper adjustment of any one of these three factors prevention or cure of rickets may be effected.

The demonstrated therapeutic value of exposure to sunlight in case of rickets has been a reason for investigating the antirachitic potency of sunlight in various parts of the country. Colonel Roger Brooke (1), an army surgeon, suggests that ideal conditions for ultraviolet light are found in Arizona, New

Mexico, and other sunny, dry portions of the United States. In these sections of the country the ultraviolet rays from sunlight are especially accessable because of clear atmosphere, low humidity, small amount of cloudy weather and intense sunshine.

To date there are no published reports on determinations of antirachitic potency of Arizona sunshine. It is, therefore, the purpose of this investigation to determine quantitatively the biologically effective sunshine with reference to the cure and prevention of rickets in Tucson, Arizona.

REVIEW OF LITERATURE

The credit for the discovery of the antirachitic vitamin is given to the Englishman, Mellanby (2), who published reports of his work in 1919. Working with puppies he produced rickets by feeding deficient diets and with addition of cod liver oil he was able to cure the disease. He did not draw proper conclusions insomuch as he attributed the rickets curing property to vitamin A because it was a vitamin A rich substance which cured rickets in his animals. In 1921 McCollum (3) and his coworkers at John's Hopkins University furnished definite proof that the rickets curing substance was a separate and distinct factor from the growth promoting factor, vitamin A. By oxidizing it inactive and feeding the resulting product to rachitic rats they cured rickets. In 1924 Hess (4) found that the unsaponifiable residue of the vitamin D containing oils was the vitamin D active portion of the oil. This unsaponifiable fraction was investigated by Rosenheim and Webster (5) and it was decided that cholesterol was the antirachitic substance but further investigation by Hess and Wandaus (6) proved ergosterol, a contaminant of cholesterol was the real parent substance of vitamin D.

The first recognized association of exposure to ultraviolet light of sunlight or quartz mercury lamp to the healing of rickets was made by Huldinsky (7) in Berlin in 1919. His work with severely rachitic children which he gave radiations from sunlight or quartz mercury lamp, demonstrated rapid and complete

healing of affected bones.

Hess and Unger (8) have confirmed the therapeutic value of sunlight in the cure of rickets in infants and also in laboratory animals.

Hess (9) in New York and Steenbock (30) at the University of Wisconsin (1924) independently and simultaneously report that inert foods containing cholesterol or closely allied substances were activated by irradiation with ultraviolet light.

Hess (4) and co-workers in the same year while reporting on the activation of cholesterol suggest that since the skin contains much cholesterol close to the surface, that ultraviolet irradiation must effect antirachitic action through this medium. Recently Knudson (10) (1932) investigated the amount of ultraviolet radiation necessary to cure rickets in rats in respect to area of skin exposed to source of ultraviolet. He found that in exposing only a small part (1/80) of total area of the rat gave complete healing in a short time.

Laurens (11) quotes Knipping, an investigator in the tropics, as reporting that the maximum intensity of the ultraviolet in the British and Dutch Indies is reduced markedly by humidity and dust.

It has been found by Mayer (12) in his investigation of intensities of ultraviolet measured at different altitudes that the ultraviolet is limited with respect to the amount of atmosphere through which it must pass. Also, there are conditions of the atmosphere which cut out the shorter wave lengths, that is higher degrees of humidity, smoke or dust.

Dr. Bundeson and Dr. Lemon (13) of Chicago report that the amount of ultraviolet absorbed or cut off by the smoke in the atmosphere as shown by their results with the spectroscope is definitely significant. On the basis of this investigation they report that very little ultraviolet radiation of "known physiologic significance" appears during the winter months in their city.

Dr. W. D. Fleming (14) who used rats testing the antirachitic potency of sunlight in the outskirts of Washington, D. C., concluded that winter sunlight was adequate for the prevention of rickets in the animals to which it was available all day long. Tisdall and Brown (15) utilizing winter sunlight in Toronto found definite antirachitic effect when the exposure period was $2\frac{1}{2}$ hours. Webster and Hill (16) in England found that a minimum of 2 hours of bright sunshine was necessary to protect rats from rickets.

Hess (17) reports that in New York September sunlight for 15 minutes per day was protective. Knudson (18), also of New York, reports that in summer it requires over 20 minutes exposure to sunlight to prevent rickets and ten times as much is required in winter. Stein and Lewis (19) report that 3.75 minutes exposure per day to Colorado sunshine during June and July is somewhat protective and from 9 to 11 minutes is almost completely protective. Day (20) in his work on Arkansas sunshine found that 10 minutes daily exposure during June and July prevented rickets but that in January and February $2\frac{1}{2}$ hours daily exposure did not prevent rickets.

Laurens and Mayerson (21), testing New Orleans sunshine, found that from the months of April to November daily exposure of 2 to 3 minutes prevents rickets in rats and from the months of November to March it requires 5 to 6 minutes.

A number of methods for testing for the antirachitic vitamin have been developed and used by different workers in the field. McCollum (22) in studying rickets made histologic examinations of the bones of the rachitic animals. He found upon immersing the split head of the tibia into 1% silver nitrate and exposing to light that the newly deposited calcium showed up as black material. Upon microscopic examination the degrees of calcification may be determined quite accureately.

Bills (23) has further developed the line test technique and suggests expression of results of quantitative vitamin D determinations in terms of degrees of healing and degrees of rickets.

More recent studies have been made by Knudson and Moor (24) in which they used radiographic pictures to confirm their line test findings. They consider the line test the more sensitive of the two.

Bethke, Steenbock, and Nelson (25) report that with varying vitamin and mineral intake there is a related variation in blood phosphates, blood calcium and ash content of bones. However, the latter variation is consistent with the severity of rickets noted and the former is not. They attempted to use growth as a criteria for judging vitamin D content of food but

failed, because the lack of vitamin A in suffecient amounts in the basal diet, the failure to gain was not due to vitamin D deficiency alone.

Chick, Korenchevsky, and Roscoe (26) use the "A/R ratio" which is ash to organic residue in expressing the degree of rickets, which is another type of ash determination. Sherman and Hessler (28) determined the degree of calcium deposition by the percentage calcium of total body of the animal.

Hart and Steenbock (27) have found that by balance experiments the calcium retention could be measured and thus the antirachitic factor measured quantitatively.

EXPERIMENTAL PROCEDURE

General Plan of Experiment

The antirachitic potency of sunlight may be determined in terms of the minimum quantity necessary to prevent rickets in rats on a rickets producing diet or as the minimum quantity necessary to produce a definite curative effect on rachitic rats. In this investigation, in which the antirachitic potency of Tucson sunshine was measured, both the curative and preventive types of experiments were used.

The antirachitic potency of Arizona sunshine was tested in Tucson, the location of which is Lat $32^{o}14'$ N. Long $7^{h}23^{m}43^{s}$, with an altitude of 2,482 feet.*

The investigation of the rickets curing power of the sunlight paralleled the study of the rickets preventing power of the sunlight. Recognizing seasonal variation in the antirachitic potency of sunlight both types of experiments were repeated or carried on for each month separately during October, November, December, January, February, March, and April. Weather records including observations of such factors which alter the antirachitic potency of the sunlight were kept. This data is tabulated below.

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* By the courtesy of Doctor A. E. Douglas, Professor of Astronomy, University of Arizona.

Months	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Relative Humidity Percent	45.6	34.2	49.0	38.3	41.0	27.6	26.0
Temperature Mean - ^{o}F	72.0	59.0	52.2	49.3	56.3	63.9	68.6
Av. Wind Velocity Miles/Hr.	7.0	7.3	5.5	6.6	6.7	6.7	6.9
No. Cloudy Days Per Mo. (At time of exposure)	5	4	4	1	5	0	3

The exposure to sunlight was made on the top of the Agricultural building on the University of Arizona campus. The roof of this building, three stories high, made an ideal place to irradiate the animals because of lack of dust from traffic and other factors which could not have been controlled otherwise. The irradiation was begun at 11 o'clock A. M. , this being the time of day just before the ultraviolet light from solar radiation is at its average maximum.

Source and Care of Experimental Animals

The rats used in this experiment came from the stock colony which is maintained on Sherman's Diet B (29) consisting of 2/3 whole wheat, 1/3 whole milk powder, and Na Cl 2% of wheat plus meat scraps. The litters were separated from the mothers at four weeks or when they reached the proper weights. Only animals weighing from 48 to 68 grams were used. The litters were put on Steenbock's rickets producing diet

Number 2965 which contains:

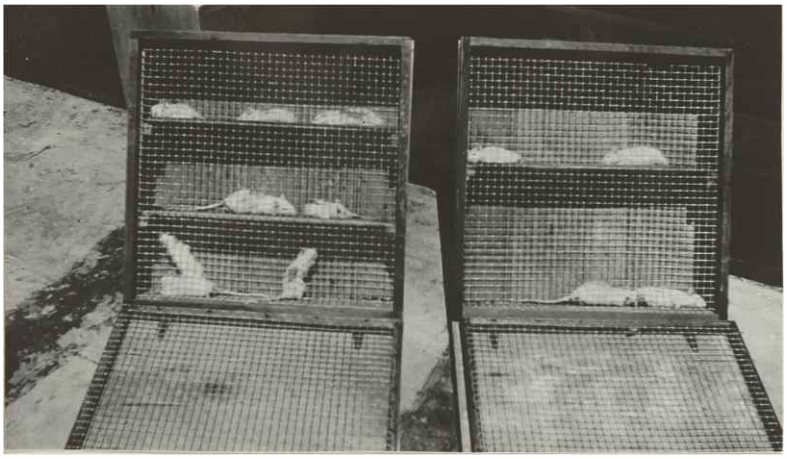
Yellow Corn	760 grams
Wheat Gluten	200 grams
Calcium Carbonate	30 grams
Sodium Chloride	10 grams

After the first month calcium carbonate content was increased to 4% which made the rickets producing effect of the diet more severe due to the more severely upset Ca:P ratio of the diet.

The animals used on this experiment were kept in as nearly total darkness as possible, that is, in a room with window shade drawn completely and electric lights shaded so that their rays did not strike the cages. The lights were used only when the rats were being cared for. With these precautions the rats, after being put on the experiment, received virtually no exposure to any light except for the controlled exposures to sunlight.

Wooden boxes 2' x 2' x 4" with 9 ½" air holes bored in top and sides were used for transporting and exposing the animals. The rats were placed in these boxes in the dark room and carried to the roof. The boxes were lined up along a wall and set at such an angle that the whole inside of the box was exposed to sunshine. The lids were dropped and wire gauze screens of ½" mesh were put over the front of the boxes to keep the animals inside. These boxes had shelves in them which made it possible to have not more than 4 or 5 rats on a shelf which allowed room for complete irradiation of each animal. The animals were carefully watched and if any crowding together occurred they were scattered to insure the maxi-

mum exposure of each rat to the sunshine. At the end of the exposure period the screens were removed, the boxes closed quickly and the rats returned to their cages in the dark room.



Showing Manner in Which Animals Were
Exposed to Sunlight

The animals used for testing the rickets preventing power of the sunshine were separated at weaning into groups which received various lengths of exposure to direct sunlight. The periods of exposure to sunlight range from 1 to 15 minutes per day. Two animals from each litter served as negative controls. Weekly weight records were kept.

These animals were irradiated for 3 to 4 weeks, or until the negative controls showed severe rickets. All the animals were then killed and tested for rickets in a manner to be described.

The animals used in the determination of the rickets curing power of sunlight were treated in exactly the same ways as the preventive group with the exception that they were

allowed to develop rickets before the exposures were begun. They were separated into groups, each of which received definite amounts of daily exposure to sunlight, ranging from 5 to 30 minutes. At least four and sometimes 6 or 8 animals were used for each exposure period. The animals were weighed every 5 days and weights recorded. If any animal showed below average gain as compared with its litter mates it was disposed of because of chance of healing through starvation. These groups of rachitic animals were irradiated at the same time and under the conditions as were the animals on the preventive experiment.

After the exposures were continued for 5 days, two animals on the highest level of exposure, that is, those receiving the most sunlight, were killed and the degree of healing of the rachitic bones noted. If they showed advanced healing, the rest on that level were killed and the animals on the next lower level tested. When the animals tested showed only slight or no healing the remainder of the animals were exposed as before for another five day period and similiar tests made.

Methods for Testing Rickets

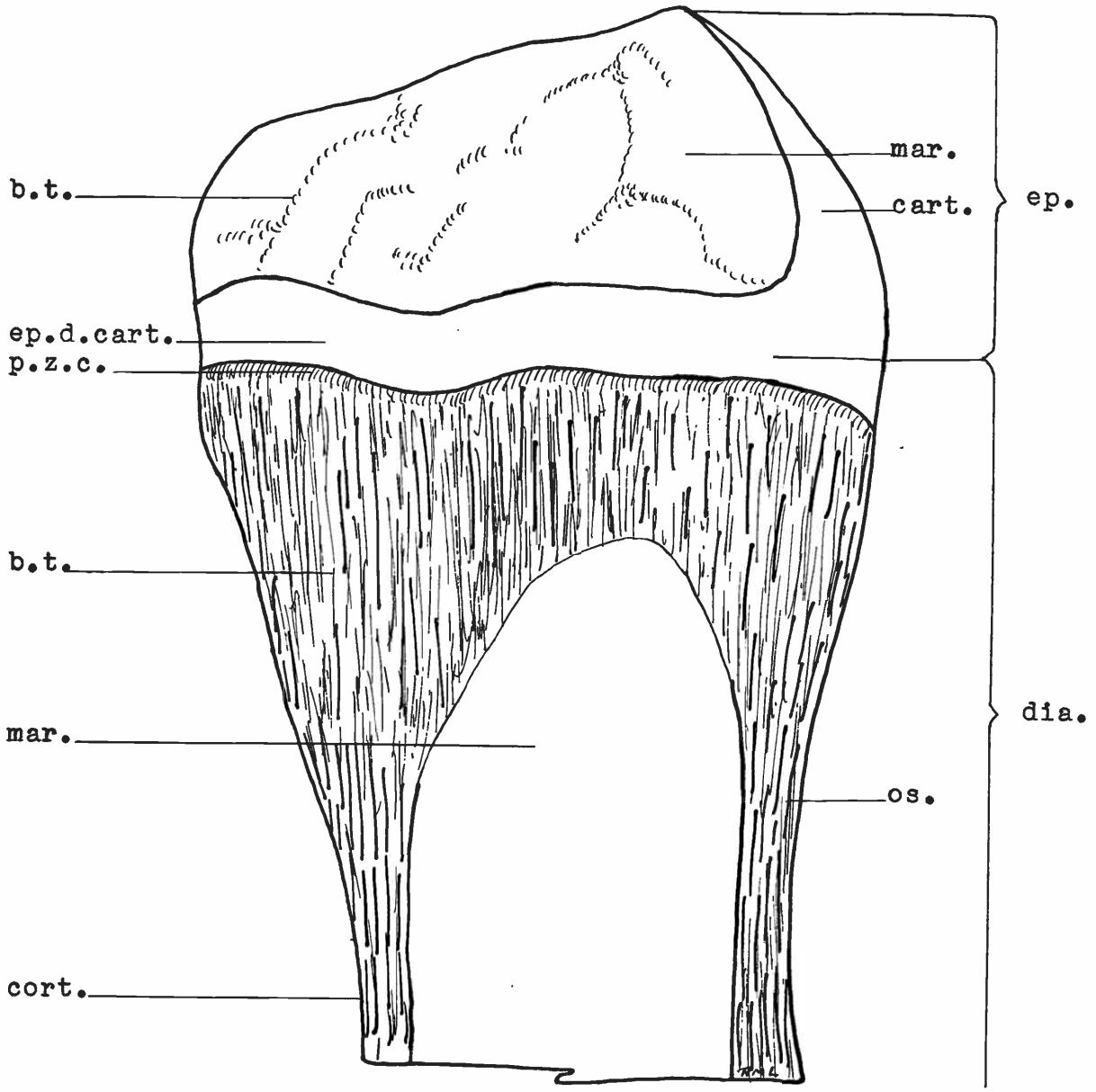
There are four methods commonly used for testing the antirachitic potency. These methods include the following: chemical analysis of ash content of bone, chemical analysis of calcium and phosphorus content of blood, x-ray examination of bone, and microscopic examination of the bone (line test).

The line test (22) used in judging the severity of rickets,

is a very delicate, more or less quantitative estimate of the amount of calcium in the bone. In rickets there is a lack of calcification in the bone which presents an abnormal histologic structure which is readily detected by the line test. This test consists of removing the tibia, cutting the bone lengthwise through the head, washing out the superfluous blood in distilled water and immersing the sections of the bone, cut side exposed, in 1% silver nitrate solution. A silver phosphate is formed on the calcified areas and this, upon exposure, is reduced to black silver. Thus, with use of the microscope, the degree of calcification or decalcification can be seen very easily.

With the animals on the preventive experiments results are expressed as normal bone, one positive (+), two positive (++), three positive (+++), or four positive (++++) rickets, the range from one positive to four positive rickets representing from slight to very severe decalcification as shown by the amount of black silver deposited on the bone. The degree of rickets suggested by one positive (+) rickets is slight, the bone showing a swollen epiphysis and a slight lowering of the zone of calcification. Two and three positive rickets simply mean more and more rachitic, being the regular intermediate steps to four positive rickets. In the most severe type of rickets the epiphysis is very wide and irregular and the metaphysis contains practically no calcified areas. These various degrees of rickets are represented by drawings in Figure II.

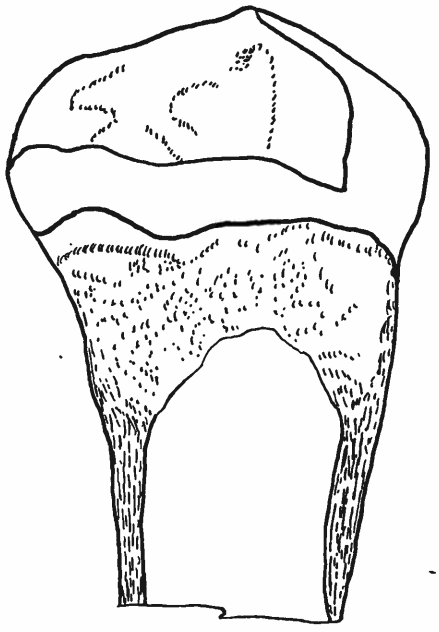
FIGURE I
NORMAL BONE AS SHOWN BY LINE TEST



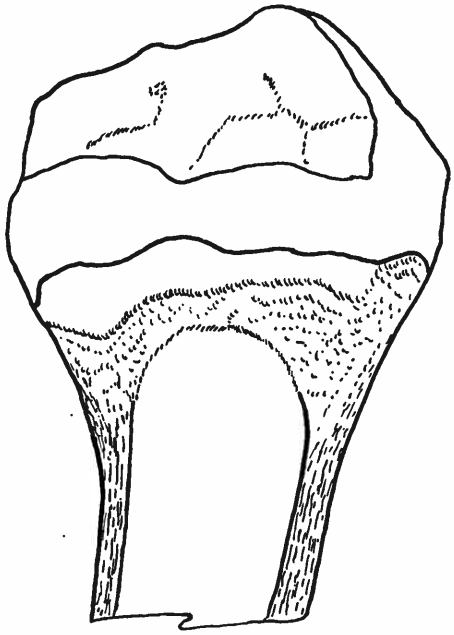
LEGEND

b.t. - bony trabecula
cart. - cartilage
dia. - diaphysis
ep. - epiphysis
ep.d.cart. - epiphysial disc of cartilage
p.z.c. - provisional zone of calcification
cort. - cortex
mar. - marrow
os. - osteoid

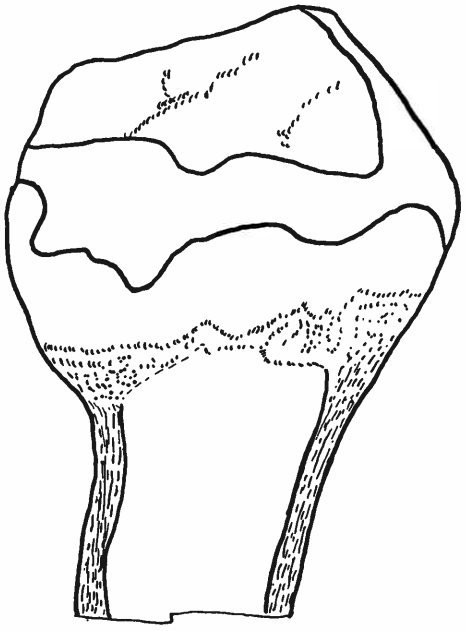
FIGURE II
SHOWING FOUR DEGREES OF RICKETS



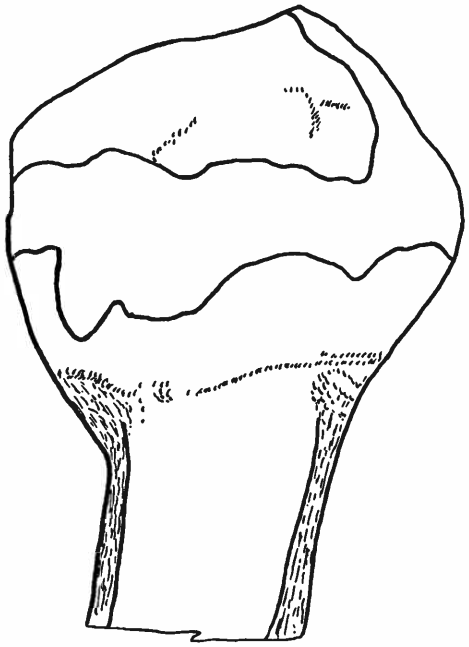
+ Rickets



++ Rickets

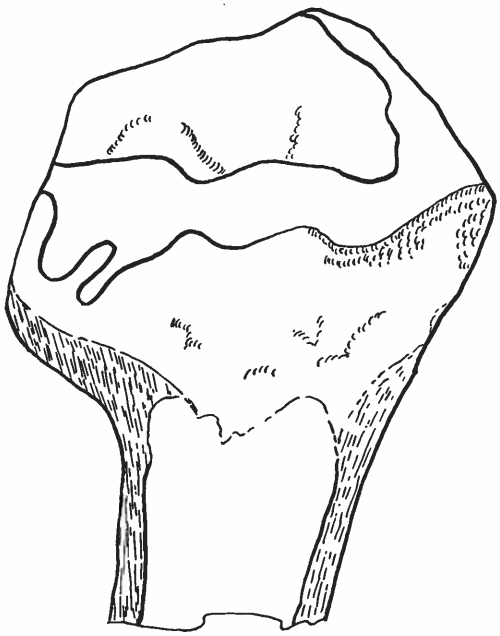


+++ Rickets

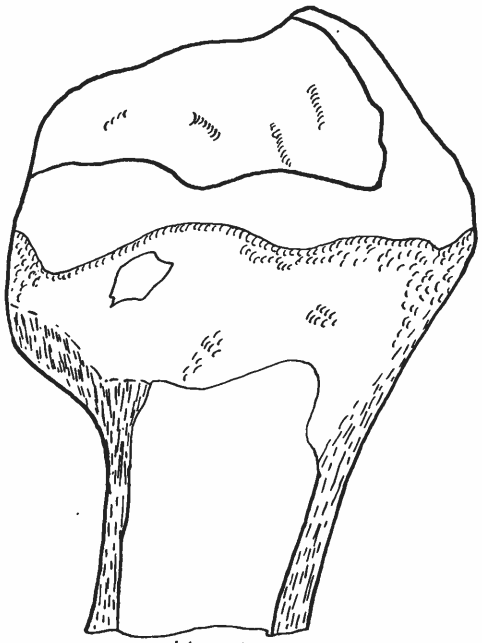


++++ Rickets

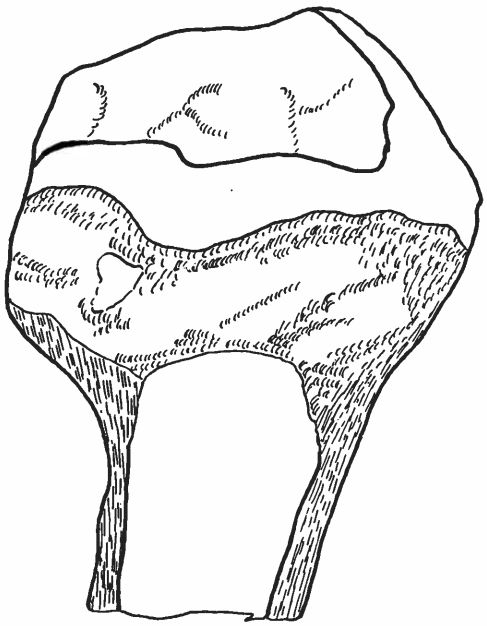
FIGURE III
SHOWING FOUR DEGREES OF HEALING



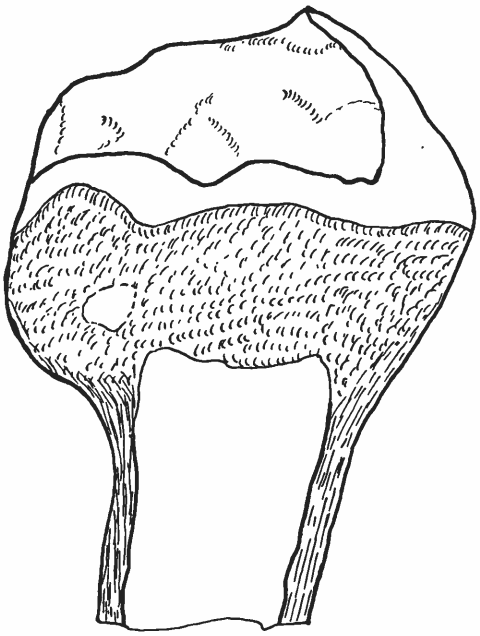
+ Healing



++ Healing



+++ Healing



++++ Healing

The degree of healing in the animals on the curative experiment is expressed in terms of one positive to four positive healing. Calcification in the rachitic bone takes place first at the top (head end) of the metaphysis. Some very scattered calcified areas may appear all over the metaphyseal band but even so, a very slight degree of healing is easily detected. One positive healing may be interpreted as being a narrow line of calcification partially across the top of the metaphyseal band and perhaps a few calcified areas below the line of calcification. A bone with a narrow epiphysis and a wide but well calcified metaphysis is represented as showing four posotive healing. Figure III shows examples of each of the stages of healing which may be found.

Healed rachitic bones do not approach the normal either in gross appearance or in comparison by the line test. However, in total ash content they may approximate the normal bone because the organic to the inorganic ratio in the rachitic bone is more or less corrected in the healing process. As may be noted by comparison of Figures I and III there are three ways in which the normal tibia differs from the healed rachitic tibia, the latter having a much larger head, a more irregular epiphyseal disc of cartilage and a much wider metaphysis than the normal bone.

In determining total ash the bones were extracted with 95% alcohol and ether, two nine hour periods for each. The bones were put in crucibles in a drying oven at 100° C for seven days. At the end of this time they were weighed, (this

weight serving as dry weight of the bone) and were ashed in a furnace at 900° C for 48 hours. They were again weighed and percentage of ash in the dry bone calculated from the data.

RESULTS AND DISCUSSION

The antirachitic potency of Tucson sunshine as measured in terms of amount of sunshine in minutes per day necessary to cure rickets in rats during the months of October to April is shown in Tables I to VII inclusive.

Table I - Measurement of the Amount of Sunshine Necessary to Heal Rickets in October

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
9947	96	+2	5	0	+++R	23
9946	113	+10	5	5	-H	27
9944	96	+6	5	10	-H	26
9998	100	+2	5	10	-H	26
9991	94	+2	5	15	-H	26
9993	91	+2	5	20	-H	32
9948	80	+16	10	0	+++R	22
9995	110	+14	10	0	+++R	22
10002	100	+2	10	0	+++R	22
9949	119	+13	10	2.5	-H	24
9951	66	+4	10	5	-H	24
9941	102	+6	10	10	++++H	46
9954	80	+18	10	10	++++H	47
9938	108	+2	15	0	++++R	27
9937	104	+7	15	2.5	-H	25
9939	104	+6	15	5	+H	31
9994	100	+10	15	5	++H	29
9997	77	+13	15	10	+++H	46
9999	100	+3	15	10	++++H	48

It may be noted from data presented in Table I that during October no healing occurred during a 5 day period regardless of the length of daily exposure to sunlight up to twenty minutes.

When the length of period was increased to 10 days neither 2.5 minutes nor 5 minutes daily exposure produced any healing. On the other hand 10 minutes exposure to sunlight for a period of 10 days completely healed the rachitic animals. The period of days was increased to fifteen and the exposure of 2.5 minutes per day produced no healing. With the fifteen day period 5 minutes exposure to sunshine caused considerable healing and 10 minutes caused practically complete healing. The shortest length of time required in the month of October to prcduce complete healing is 10 minutes per day exposure for 10 days.

Table II - Measurement of the Amount of Sunshine Necessary to Heal Rickets in November

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10122	108	+10	5	0	++++R	21
10123	90	+4	5	5	-H	26
10127	106	+9	5	10	-H	24
10124	100	+11	5	15	-H	33
10136	110	+11	5	15	-H	33
10141	82	+8	5	20	-H	32
10170	70	+14	10	0	++++R	23
10168	70	+20	10	5	-H	26
10145	93	+9	10	5	-H	26
10137	104	+16	10	10	-H	25
10146	80	+6	10	15	-H	39
10141	96	+7	10	20	++++H	41
10142	94	+14	10	20	++++H	44
10147	80	+10	10	20	+++H	44
10140	100	+19	15	0	++++R	27
10131	72	+12	15	15	+H	31
10135	70	+14	20	0	++++R	26
10145	90	+27	20	0	++++R	26
10132	74	+30	20	5	-H	28
10138	78	+9	20	5	-H	28
10166	85	+19	20	10	+++H	49
10134	79	+15	20	10	+++H	49
10165	74	+12	20	15	++++H	47
10160	86	+7	20	15	++++H	47

Table II shows that during the month of November, also no healing occurred in a 5 day exposure period, even when exposure in minutes per day was increased to twenty. In a 10 day period neither 5, 10 nor 15 minutes exposure per day produced any healing, but 20 minutes per day proved to be curative. When the length of period was increased to 15 days 15 minutes exposure time was somewhat curative. On a 20 day period 5 minutes exposure per day produced no healing. Ten minutes was definitely curative (+++H) and 15 minutes healed completely.

It may be noted that healing does not take place directly in proportion to the total number of minutes exposed. That is, in the case of the 10 day period with 20 minutes exposure to sunshine (a total of 200 minutes) produced complete healing, but when the period was 20 days with 10 minute exposure time (again a total of 200 minutes) complete healing did not result. With 20 days on 15 minutes (300 minutes total) exposure a complete cure was effected.

Table III, results for December, shows no healing in 10 day periods with 15 and 20 minutes per day exposure to sunshine. No appreciable healing was evidenced in the 15 minutes exposure for 15 days, but when the exposure time was lengthened to 20 minutes it produced advanced healing. Using the 20 day period, 10 minutes exposure time produced varied degrees of healing and 15 minutes exposure caused advanced healing. Twenty minutes for 20 days effected complete healing which was five minutes more than in November for the same number of days.

Table III - Measurement of the Amount of Sunshine Necessary to Heal Rickets in December

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10336	78	+6	10	0	++++R	26
10301	106	+14	10	15	-H	28
10337	68	+9	10	15	-H	28
10300	100	+16	10	20	-H	37
10312	78	+23	10	20	-H	37
10304	82	+22	15	15	-H	37
10313	74	+19	15	20	+++H	42
10322	78	+22	15	20	+++H	42
10303	78	+6	20	0	++++R	22
10308	100	+12	20	0	++++R	22
10315	110	+10	20	0	++++R	22
10310	78	+10	20	10	-H	39
10318	126	+20	20	10	++H	39
10319	100	+12	20	10	++H	39
10306	84	+18	20	10	+++H	42
10335	76	+10	20	10	+++H	42
10327	80	+20	20	10	++++H	46
10309	94	+20	20	15	+++H	46
10334	73	+7	20	15	++++H	47
10311	78	+34	20	20	++++H	47
10317	109	+21	20	20	++++H	47

During the month of January (Table IV) a 10 day period with 20 and 30 minutes exposure to sunlight produced definite but not complete healing. In 15 days 20 minutes exposure produced advanced healing. Ten minutes for 25 days produced complete healing also. Here again was an example of healing not in proportion to the total time exposed; with the 15 day period the total time required to heal completely was 450 minutes, with 20 days 400 minutes, and with 25 days 250 minutes.

Table IV - Measurement of the Amount of Sunshine Necessary to Heal Rickets in January

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10500	81	+7	10	0	++++R	23
10483	90	+6	10	20	+H	29
10489	90	+14	10	30	++H	38
10493	86	+10	10	30	+++H	38
10466	74	+14	15	20	+++H	38
10499	98	+8	15	20	+++H	38
10482	76	+16	15	30	+++H	44
10497	100	+10	15	30	+++H	44
10465	86	+4	15	30	++++H	45
10504	88	+4	15	30	++++H	45
10490	96	+10	20	15	++H	44
10481	98	+11	20	15	+++H	44
10470	84	+20	20	15	+++H	42
10495	78	+14	20	20	++++H	47
10468	72	+18	20	20	++++H	47
10467	72	+28	25	0	++++R	22
10485	82	+10	25	0	++++R	22
10494	78	+14	25	0	++++R	22
10484	98	+4	25	10	+H	37
10469	72	+25	25	10	++H	39
10491	80	+28	25	10	++++H	43
10492	80	+22	25	10	++++H	43

February presents very much the same results as January: 30 minutes exposure to sunshine for a 10 day period does not heal completely nor does 20 minutes for 15 days. When the length of period in days is 20, 10 minutes, 15 minutes and 20 minutes exposures do not cause complete healing. As in the preceding month, when the period in days is 25 and the minutes per day exposed is 10 (250 minutes total) complete healing is evidenced.

Table V - Measurement of the Amount of Sunshine Necessary to Heal Rickets in February

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10691	64	+2	10	30	+++H	40
10694	66	+4	10	30	+++H	40
106828	64	+8	15	0	+++R	24
10695	68	+2	15	20	++H	36
106824	64	+2	15	20	+++H	36
106827	70	+7	15	20	+++H	36
106823	62	+18	15	20	+++H	37
106821	64	+6	20	10	++H	38
10625	60	+4	20	10	++H	38
10690	60	+10	20	10	++H	38
10696	70	+4	20	15	+H	34
10698	62	+3	20	15	++H	37
106830	74	+2	20	15	++H	37
106820	67	+6	20	15	+++H	40
106817	86	+6	20	15	+++H	40
106822	66	+2	20	20	+++H	46
106818	76	+4	20	20	++++H	49
106816	78	+6	25	0	++++R	23
10693	68	+10	25	0	++++R	23
106826	70	+8	25	10	+++H	37
10692	50	+18	25	10	+++H	37
10697	68	+4	25	10	+++H	37
106819	64	+6	25	10	++++H	46
106823	66	+10	25	10	++++H	46

March shows an increase in rickets healing ability over the past three months. Ten days of 20 minutes daily exposure to sunlight shows marked healing and 15 days with 20 minute exposure shows almost complete healing. Twenty days with 10 minute exposure (total time 200 minutes on longer exposure period) heals entirely.

Table VI - Measurement of the Amount of Sunshine Necessary to Heal Rickets in March

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10805	72	+6	10	0	++++R	23
10806	78	+6	10	20	+H	25
10811	60	+4	10	20	+H	29
10810	60	+2	10	20	-+++H	32
10804	61	+4	15	10	++H	32
10822	64	+4	15	15	+++H	37
10823	64	+6	15	15	+++H	37
10818	78	+6	15	20	++++H	46
10800	72	+4	15	20	+++H	44
10820	60	+8	15	20	++++H	46
10821	64	+6	15	20	++++H	46
10807	70	-+5	15	20	++++H	46
10803	72	+6	20	0	++++R	20
10824	68	+4	20	0	++++R	20
10802	80	+6	20	10	+++H	38
10809	62	+6	20	10	++++H	44

The record for April shows that in a period of 10 days with 15 minutes daily exposure to sunshine, the rachitic bones were completely healed, which shows a decided increase in the healing ability of the sunshine of April over that of March.

With the animals which received 10, 15, and 20 minutes exposure during a period of 15 days we find variable results, but these, as in the case of those exposed for 10 days, indicate increased antirachitic potency of sunshine over previous months. If this 20 day period is used, 10 minutes is the amount of time necessary to cause complete healing of the rachitic animal.

Table VII - Measurement of the Amount of Sunshine Necessary to Heal Rickets in April

Rat No.	Experimental Period				Line test findings	Ash content of bone %
	Wt. at end of fore-period grams	Wt. change during period grams	Length of period days	Exposure per day mins.		
10964	68	+8	10	0	++++R	23
10972	58	+4	10	10	+H	29
10967	62	+12	10	10	+++H	37
10962	74	+10	10	15	++++H	46
10971	68	+4	10	15	+++H	46
10925	74	+6	10	20	+H	34
10968	72	+6	15	10	+++H	38
11002	72	+8	15	10	++++H	38
10929	74	+6	15	15	++++H	47
10923	62	+8	15	15	+++H	47
10960	76	+5	15	15	++++H	47
10926	70	+11	15	20	++++H	47
10965	72	+8	15	20	+++H	47
10927	72	+8	15	20	++++H	47
10966	74	+6	15	20	++++H	47
10930	70	+2	20	0	++++R	20
11000	96	+3	20	0	++++R	20
11020	72	+14	20	0	++++R	20
11029	58	+20	20	10	+++H	42
11006	68	+13	20	10	++++H	48
11026	65	+3	20	10	++++H	48

The results of the ash analysis expressed as total ash of dry weight of bone confirm the line test findings. The rachitic bones show a very low value of 20 to 27% total ash. In cases of healing this percentage increases with the degree of healing, from 31% in slight healing to 49% in completely healed bone.

In order to compare findings during the various months when the curative method of testing antirachitic potency of

sunlight is used, it is necessary to express the results on a comparable day basis, as is shown in summary Table A.

Table A - Amount of Sunshine in Minutes per Day for Ten, Fifteen, and Twenty Days Necessary to Produce Complete Healing of Rickets in Rats for the Months October to April, Inclusive

Days	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
	minutes per day						
10	10	20	(20+)'	30+	30+	20+	15
15	10	(10+)'	20	30	20	15	15
20	*	15	20	20	20	10+	10

'No complete healing in animals exposed for that number of days

*No animals exposed for that period of time for that particular month

The above, Table A, summarizes the results for those months and shows that on a 10, 15, or 20 day basis that the same general trend in antirachitic potency is shown; that is, the amount of sunshine in minutes per day necessary to heal rickets increases from October to January, and from January to April this amount decreases. Thesedata illustrates clearly the phenomenon of seasonal variation. The general trend as shown by the curative experiments is represented graphically in Figure 4.

These results show that in January and February, the months showing lowest antirachitic potency, three times as much sunshine was necessary to heal rickets on the same day basis as was necessary during the month of October. During April half as much sunshine was required as was necessary in January and

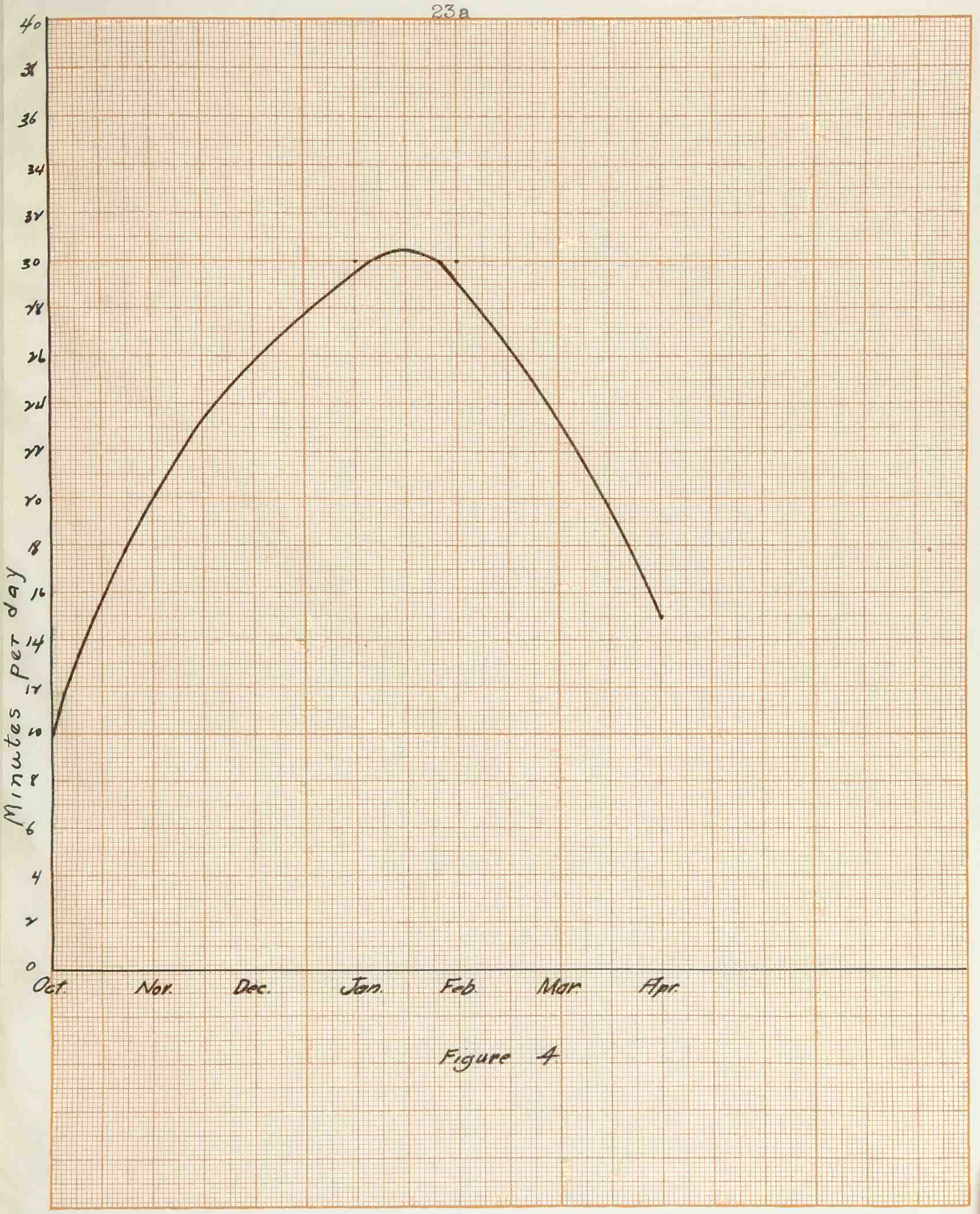


Figure 4

February. Knudson (19) reports summer sunshine in New York when tested by curative method to be 10 times as potent in rickets healing power as winter sun. Winter sunshine in Arizona shows a small difference in antirachitic potency for the seasons as compared to Knudson's findings in New York.

The amount of sunshine necessary to prevent rickets in rats expressed in minutes of exposure to sunshine per day for the months of October to April inclusive is given in Tables VIII to XIV.

Table VIII - Showing the Amount of Sunshine Necessary to Prevent Rickets During October

Rat No.	Initial Wt. grams	Av. Wt. Change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10028	54	4.0	0	+++R	27
10033	60	6.5	0	+++R	28
10037	54	6.0	0	++++R	24
10041	54	6.2	2.5	+R	31
10054	52	8.6	2.5	++R	32
10035	56	9.0	2.5	++++R	28
10020	64	12.5	2.5	++++R	21
10026	56	5.0	5	+++R	22
10029	64	8.0	5	Normal	38
10039	56	5.2	5	Normal	38
10052	54	5.2	5	Normal	38
10022	64	6.0	10	++R	34
10030	56	6.0	10	Normal	39
10040	56	5.6	10	Normal	39
10053	54	10.0	10	Normal	39
10024	60	10.5	15	Normal	48
10025	60	10.0	15	Normal	48
10031	66	11.0	15	Normal	48
10038	50	10.5	15	Normal	48

In Table VIII we may note that during October 2.5 minute exposure to sunshine per day was not sufficient to prevent

rickets in rats, but five minutes exposure per day was sufficient to produce a normal bone. The weather record shows 5 cloudy days for October, which is 11% of the total days these animals were exposed.

Table IX - Showing the Amount of Sunshine Necessary to Prevent Rickets during November

Rat No.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10198	70	8.2	0	+++R	30
10203	70	7.5	0	++++R	21
10284	56	7.0	0	++++R	21
10127	54	12.5	1	+++R	22
10280	56	10.0	1	+++R	22
10282	58	11.0	1	++++R	23
10283	58	9.0	1	++++R	23
10201	68	8.0	2.5	+R	31
10279	58	10.0	2.5	+++R	24
10281	60	10.0	2.5	+++R	22
10231	52	11.6	2.5	+++R	22
10233	50	10.5	2.5	+++R	22
10200	71	10.6	5	Normal	31
10204	74	7.0	5	++R	28
10232	52	10.5	5	Normal	30
10199	74	9.5	10	+R	32
10202	70	9.8	10	Normal	34
10229	52	10.5	10	+R	39
10197	72	8.2	15	Normal	36
10277	54	12.5	15	Normal	38

Table IX shows that for the month of November 10 minutes exposure to sunshine per day nearly prevents rickets and 15 minutes per day does prevent rickets. This is nearly three times as much sunshine as was necessary the preceding month. There was little variation in the weather, being 4 cloudy days which is 14% of total number of days exposed.

Table X - Showing the Amount of Sunshine Necessary to Prevent Rickets during December

Rat No.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10403	50	9.5	0	+++R	28
10408	58	9.5	0	+++R	28
10415	48	13.0	0	++R	27
10416	62	7.0	0	++++R	23
10399	62	7.7	2.5	++R	25
10404	50	6.0	2.5	+++R	24
10411	54	8.0	2.5	++++R	24
10400	58	9.5	5	++R	22
10410	54	7.5	5	+++R	23
10412	54	7.0	5	+++R	23
10418	66	8.1	5	+++R	23
10401	56	7.6	10	++R	24
10409	54	12.5	10	+R	33
10413	50	9.6	10	++R	38
10419	64	9.0	10	++R	38
10402	58	10.5	15	Normal	39
10406	56	9.0	15	Normal	39
10414	50	7.5	15	Normal	46
10417	58	7.0	15	Normal	46
10420	62	7.6	15	Normal	46

During the months of December and January as shown by Tables X and XI 15 minutes per day irradiation was required to prevent rickets, which is only slightly more than was necessary in November. December weather records show 4 cloudy days and January show one.

Table XI - Showing the Amount of Sunshine Necessary to Prevent Rickets during January

Rat no.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10641	50	10.0	0	+++R	20
10648	56	7.0	0	++++R	24
10655	50	8.0	0	+++R	20
10638	56	6.0	5	Normal*	32
10642	46	8.0	5	++R	28
10645	54	7.0	5	+R	30
10649	58	10.0	5	+++R	26
10652	56	7.0	5	+++R	26
10656	48	8.5	5	++R	28
10639	54	6.8	10	Normal*	39
10643	52	6.0	10	Normal	41
10646	60	9.4	10	Normal	41
10650	58	8.2	10	Normal*	38
10653	54	5.0	10	Normal	41
10657	50	8.5	10	Normal	41
10640	50	9.0	15	Normal	43
10644	48	8.5	15	Normal	43
10647	58	9.5	15	Normal	43
10651	56	6.5	15	Normal	47
10654	56	11.5	15	Normal	47
10658	50	9.0	15	Normal	47

Table XII showing the results for February shows a decided drop in the amount of sunlight necessary to prevent, as compared to the two preceding months. The minimum length of exposure per day which produced normal bone during the month was 10 minutes, which is not yet as short an exposure period as was required during October. February weather records show 5 cloudy days, or 17% of total.

* With swollen epiphysis

Table XII - Showing the Amount of Sunshine Necessary to Prevent Rickets during February

Rat No.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10782	48	5.0	0	+++R	22
10783	48	8.0	0	++R	24
10787	50	7.5	0	++R	24
10789	50	6.0	0	++++R	26
10777	56	6.0	5	S. E.*	31
10784	50	6.0	5	Normal	32
10788	50	7.5	5	++++R	27
10778	52	7.5	10	Normal	38
10779	50	7.5	10	Normal	38
10785	52	7.1	10	S. E.	39
10786	52	5.0	10	S. E.	39
10780	50	7.0	15	Normal	43
10781	48	6.4	15	Normal	43
10790	52	5.0	15	Normal	43

Table XIII - Showing the Amount of Sunshine Necessary to Prevent Rickets during March

Rat No.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
10880	60	8.0	0	+++R	22
10883	54	6.5	0	+++R	22
10886	50	7.5	0	++++R	23
10932	52	6.0	0	++++R	23
10914	52	5.5	0	++++R	23
10878	60	6.4	2.5	Normal	32
10884	54	6.4	2.5	Normal	32
10931	52	7.0	2.5	S. E.	34
10935	50	7.5	2.5	S. E.	34
10916	46	6.5	2.5	S. E.	34
10887	50	6.5	5	++++R	29
10888	38	5.5	5	S. E.	35
10911	52	6.5	5	Normal	44
10912	52	8.0	5	Normal	44
10885	50	5.0	10	Normal	47
10934	50	4.5	10	Normal	47
10917	44	7.0	10	Normal	47
10889	38	2.5	15	Normal	46
10933	50	1.5	15	Normal	46

*S. E. - swollen epiphysis

For the months of March and April there is a continued decrease in length of time necessary to prevent rickets. March weather records show no cloudy weather and April 3 days only. In March 5 minutes and in April 2.5 minutes per day of exposure to sunlight was necessary for prevention.

Table XIV - Showing the Amount of Sunshine Necessary to Prevent Rickets during April

Rat No.	Initial wt. grams	Av. wt. change per wk. grams	Exposure per day mins.	Line test findings	Ash content of bone %
11060	58	7.5	0	+++R	20
11064	52	4.6	0	++R	22
11070	60	5.3	0	+++R	22
11084	64	5.3	0	+++R	22
11087	60	4.0	0	+++R	22
11065	50	10.3	2.5	Normal	38
11068	60	10.0	2.5	Normal	38
11071	60	4.0	2.5	Normal	38
11059	60	4.6	5	Normal	41
11061	54	12.0	5	Normal	41
11074	60	3.0	5	Normal	41
11062	50	3.3	10	Normal	48
11063	52	5.3	10	Normal	48
11073	54	4.7	10	Normal	48
11083	64	7.0	10	Normal	48
11074	54	5.0	15	Normal	47
11085	64	8.6	15	Normal	47
11086	60	6.3	15	Normal	47

The results of the ash analysis parallel the line test findings showing low or near 20% total ash for rachitic animals and this percentage increasing with the rate of healing to 47% and 48% in the healed bones.

A comparison of the antirachitic potency os sunshine for the seven months tested is given in summary form in Table B.

Table B - Amount of Sunshine in Minutes per Day Necessary to Prevent Rickets in Rats for the Months of October to April, Inclusive

	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
	minutes per day						
Time required to prevent rickets	5+	10+	15+	10	10	5	2.5

Figure 5 shows the results of Table B in graph form, illustrating the general trend in seasonal variation of the ability of the sun rays to prevent rickets. The amount of sunshine necessary to prevent rickets diminishes during the months of January and February as shown by results from Table B. This drop occurs earlier in the season in Arizona than it does in Toronto (Tisdall) or Boulder.

It may be noted from a comparison of Table A and Table B that the amount of sunshine in minutes per day required to prevent rickets in animals on a rickets producing diet is much less than the amount required to heal rachitic lesions. It requires half or less than half as much daily exposure to sunshine to prevent as to cure rickets.

Table B illustrates, as did Table A (showing results for curative work), the seasonal variation in the antirachitic value of sunlight, the maximum in this case being in April. The length of exposure in April is about 1/6 of the time required in December and in October 1/3 as much is necessary as in

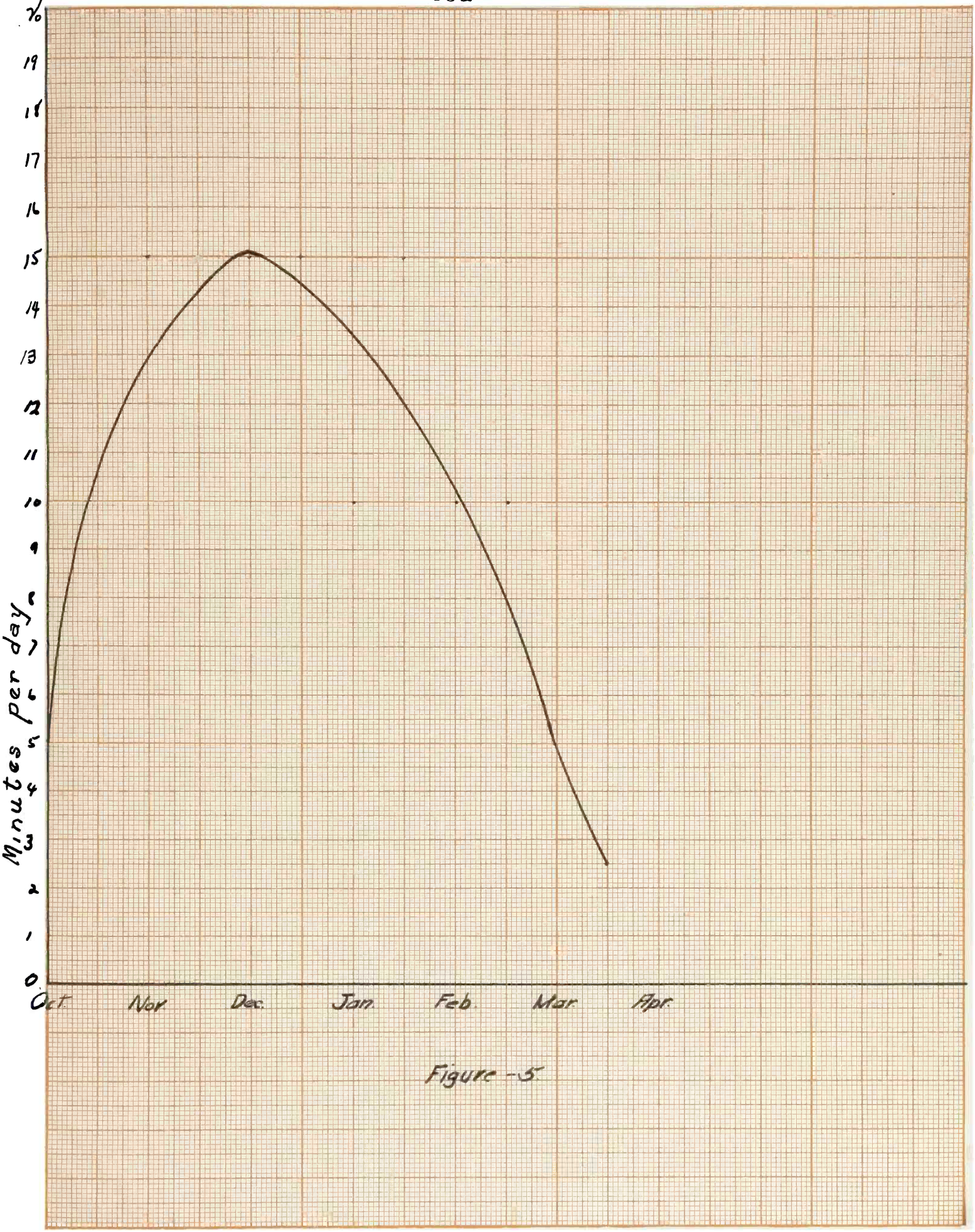


Figure -5.

December. This is not as great a difference as Tisdall found in the winter and summer sunshine in Toronto in which he reports summer sunshine to be 8 times as effective as winter sunshine in antirachitic potency. He states 15 minutes exposure to sunlight in summer and 2½ hours exposure in winter as being antirachitic. On this basis Arizona sunshine in April has 6 times the antirachitic potency of Toronto summer sun, and Arizona winter sunshine in December has 10 times the antirachitic potency of that in Toronto. It requires 15 minutes per day of New York sunshine in September to protect rats from rickets (Hess, 17) which is equal to the amount of Arizona sunshine required in December.

In comparing the antirachitic potency of Arizona sunshine with Arkansas sunshine (Day, 9) it may be noted that Arizona April sunshine is 4 times as potent as that of June and July in Arkansas. Arizona winter sunshine (January and February) has exactly 15 times the antirachitic value of January and February sunshine in Arkansas.

Colorado sunshine for June and July as reported by Stein and Lewis (19) is only ¼ as potent as Arizona sunshine in April in the prevention of rickets.

New Orleans sunshine (Laurens, 21) is preventive at 2 or 3 minutes exposure at any time between and including April and October, which is comparable to Arizona sunshine in April and October. From November till March only 5 to 6 minutes of New Orleans sunshine per day was necessary to prevent rickets and from 5 to 15 minutes of Arizona sunshine was necessary for the

same effect. The greater amount of biologically effective sunshine in Arizona is due probaby as Dr. Brooke (1) suggests to the location of Arizona, the small amount of humidity and cloudy weather, the clear atmosphere and intense sunshine.

SUMMARY

The antirachitic potency of Arizona sunshine was determined by noting the amount of sunshine in minutes per day necessary to prevent rickets in rats on Steenbock's rickets producing diet Number 2965 and also the amount of sunshine in minutes per day for periods of 10, 15, and 20 days necessary to cure rachitic animals during the months from October to April inclusive. Exposures were made from the top of a University building at 11:00 A. M. The length of time in minutes exposure per day ranged from 1 minute to 15 minutes in the preventive experiments and from 10 minutes to 30 minutes in the curative experiments.

McCollum's line test and determinations of total ash content of the tibia were used in judging the degree of rickets.

It was found that 10 minutes daily exposure healed rachitic lesions in 10 days in October and the time required for healing gradually increased until in January and the first part of February more than 30 minutes were necessary. The length of time required for healing gradually decreased to 15 minutes during the month of April. On a 15 and 20 day basis the same general trend in amount of time necessary to cure rickets was noted.

In testing the amount of sunshine in minutes per day

necessary to prevent rickets 5 minutes was sufficient to produce normal bone in rats on a rickets producing diet. This time increased to 10 minutes in October and 15 in December and decreased gradually through January and February to 5 minutes in March and finally to 2.5 minutes in April.

It is evident that during the months of October and March the antirachitic potency of sunshine was 3 times the potency in December and that during April the antirachitic potency was 6 times that during December as shown by its ability to prevent rickets in rats fed a rickets producing diet.

Summer sunshine in Arkansas shows 15 times the antirachitic potency of Arkansas winter sunshine. The ratio in antirachitic potency of summer sunshine to winter sunshine in New York is 10 to 1 and in Colorado is 8 to 1. New Orleans, however, shows a 2 to 1 ratio which is smaller in amount of variation than is shown in Arizona during the months studied.

CONCLUSIONS

It may be concluded, therefore, from all the data reported herein that Arizona sunshine has a very high biological value as evidenced by its rickets curing and rickets preventing power.

A seasonal variation in the antirachitic potency of Arizona sunshine was noted. This variation, however, is of a much smaller degree than that in other parts of the country.

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