CXXXVIII. THE DISTURBANCE OF METABOLISM PRODUCED BY BONY AND NON-BONY INJURY, WITH NOTES ON CERTAIN ABNORMAL CONDITIONS OF BONE.

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(Received May 18th, 1930.)

THE present communication is an attempt to elucidate certain of the grosser changes in the mineral metabolism of the body during the healing of a fractured bone, and to describe certain of the phenomena associated with tissue injury.

Practically all previous investigations have centred round the relative and absolute amounts of calcium and phosphorus in the blood during different phases of the healing process. The results of the various investigators in this field have been very contradictory. Tisdall and Harris [1922] state that, during the second to sixth weeks of the healing of fractures in adults, the phosphorus content of the serum is raised to about that of childhood and that there is a close parallelism between the concentration of inorganic phosphorus and the formation of new bone. This increased concentration of phosphorus begins to decline towards the normal level about the seventh week. The observations of these two workers on the question of the deviations during non-union were not decisive. Eddy and Heft [1923] confirmed this rise in phosphorus in 23 fracture cases, but found a similar rise in 20 cases involving surgical operations and the healing of tissues other than bone. Moorhead *et al.* [1925] examined a series of 30 fractures. In less than 50 % there was a rise to 5 mg. per 100 cc. serum. Union in some cases was observed with practically no increase of the phosphorus content and non-union in other cases with a high figure. Several cases showed slight variations in the serum-calcium paralleling the phosphorus. György and Sulger [1925] found that the normal phosphorus content of 3 mg. per 100 cc. rises to 5-9 mg. during the healing of a fracture, returning to normal after consolidation. When a fracture failed to unite they found that the phosphorus content had not risen. They also found an increase of phosphorus after osteotomies but not after operations on the soft parts. Satanowski [1925] found in dogs a rise of about 1 mg. 3 days after a fracture. The calcium content remained rather irregular, but slightly below normal. Henderson, Noble and Sandiford [1926] examined 20 cases of un-united fracture where the inorganic phosphorus tended to be about the normal adult content. In two cases only

did it rise to the infancy level. The blood-calcium remained normal. Petersen [1924] studied the calcium and phosphorus of the serum in 9 cases of normally healing fractures, and in 9 un-united cases. This investigator states that the union of a fracture does not occur if the product of $\text{Ca} \times \text{P}$ be less than 30. For active healing he considers that the product must lie between 35 and 40. Seven of his un-united cases went on to solid bony union on administering the defective element. They required about 3 weeks' treatment before the blood-serum showed an increase in the low salt. As a control, a similar number of normal fractures was studied: during the process of union there was little or no increase in the serum-calcium or -phosphorus. On the other hand, Ravdin and Jonas [1926] found that 5 un-united fractures gave a $\text{Ca} \times \text{P}$ product of 30-46, and 10 un-united cases gave products varying from 32-59. The blood estimations were performed 6-12 weeks after the injury.

Reviewing the work on blood analyses, the following points appear to emerge from this rather contradictory mass of evidence.

(1) That there probably is a rise in the plasma- or serum-phosphorus following a fracture, and that later this declines towards the normal level.

(2) That such a rise is not peculiar to fracture cases, but may occur after injury to parts other than bone.

(3) That the serum-calcium remains practically constant during the healing process.

As it appeared likely that further investigations along similar lines would yield little evidence of value, it was decided to undertake a series of metabolic examinations of the mineral exchange in cases of normally healing fractures, in fractures treated with irradiated ergosterol, in un-united fractures, in fractures complicated by bony abnormalities, and in wounds of non-bony parts.

GENERAL PLAN OF THE EXPERIMENTS.

In the majority of the fracture cases the metabolic data were collected during two periods. As it was generally the third day following the accident before the patients were admitted to the experimental ward, and as 3-4 days were then allowed on constant diet before the collection of excreta was commenced, the earlier metabolic periods date from the sixth or seventh day following the accident. The period of collection was 6-7 days. 5-9 weeks later a similar period was studied.

The daily excretion of a few cases of fracture and non-bony injury was examined as far as possible from the date of the accident. All the fractures were simple with the exception of case 15.

In addition to the fracture cases several other pathological conditions of bone have been examined and the metabolic data are described in so far as they throw light on certain of the phases of calcium metabolism.

Determinations of the inorganic phosphorus, non-protein-nitrogen and urea of the blood were made on a few cases of fracture and on a few cases of non-bony injury.

The daily intake of food was selected from the following list:

Dried milk (made up in fluid form from "Cow and Gate" half-cream dried milk).
 Brown bread.
 Digestive biscuits.
 Fresh butter.
 Steak (served as stew).
 Dried apples (served stewed).
 Dried tomato soup (served as soup).
 Dried egg (served as custard).
 Tea.
 Sugar.
 Marmalade.
 Orange juice.

Varying amounts of all these items were consumed.

With the exception of cases 18, 21, 32 and 34 the subjects were males.

ANALYTICAL METHODS.

The subjects of these experiments were generally in good health apart from their tissue injury. They were allowed to select the quality and quantity of foodstuffs required from a prepared list. This intake, as well as the intake of water, was kept as constant as possible. The urine was collected in 24-hourly specimens, made up to a constant volume and preserved with thymol and chloroform. The various periods during which faeces were collected were marked off with carmine, taken in the form of capsules. The faeces were dried on a steam-bath and in an air-oven before being stored in sealed bottles. The intake of mineral matter was determined at first by summation of the individual food values, but later an average half-day's intake was dried and analysed.

(a) Urine:

Total nitrogen	Kjeldahl method
Urea	Urease method
Ammonia and creatinine	Folin's methods
Total sulphur	Denis's modification of Benedict's method
Total phosphorus	Neumann's wet ash method
Calcium	Shohl and Pedley's method [1922]

(b) Faeces and food:

Total nitrogen	Kjeldahl method
Phosphorus	Material ashed in the presence of excess Ca(OH)_2 : phosphorus precipitated as ammonium phosphomolybdate and weighed directly

(b) Faeces and food (*contd.*):

Calcium	Material ashed, any iron removed, then Shohl and Pedley's method applied
Sulphur	Preliminary oxidation with nitric acid and bromine. Then Denis's modification of Benedict's method applied

(c) Blood:

Non-protein-nitrogen and urea	Folin and Wu methods
Inorganic phosphorus	Recent modifications of the Bell and Doisy technique

ANALYTICAL DATA.

The actual data only will be described here, all discussion being delayed till later. It is necessary to bear in mind that, whereas the bony skeleton contains approximately 97–98 % of the total calcium of the body, it only contains two-thirds of the total phosphorus; the remaining one-third is to be found in the muscles, glandular organs, etc.

A. *Normally healing group of fractures* (1–7) (*vide* Tables I and II).

Calcium. The urinary excretion of CaO varies from 0.0066 g. per kg. per day (p.k.p.d.) to 0.0128 g., with a mean value of 0.0092 g. during the second week of healing; and from 0.0057 g. to 0.0103 g. with a mean value of 0.0087 g. during the fifth to ninth weeks. There is a degree of constancy in this urinary excretion and such variability as exists does not appear to bear any relationship to the intake as defined by the range 0.029 g. to 0.048 g. p.k.p.d., and by a similar range during the second period.

Givens [1918] found that in healthy adults on diets containing more calcium than magnesium, the urinary output of CaO varied from 0.17 g. to 0.66 g. per day. Nelson [1920] stated that in adult men the urinary excretion of CaO varies from 0.12 g. to 0.58 g. per day when the diet is unrestricted. Unfortunately the weights of the subjects of these experiments are not recorded. On the assumption that the average weight would be about 70 kg., the maximum excretion of CaO would be approximately 0.009 g. p.k.p.d., a figure corresponding to the average value of the fracture group. Data are presented here of a few normal subjects (group G) selected from various experimental groups. It is unfortunate that the daily intakes 0.017 to 0.029 g. p.k.p.d. are considerably less than in the fracture group. The urinary excretion of CaO varied from 0.0019 to 0.0057 g. p.k.p.d. with an average of 0.0043 g. These values are distinctly lower than for the fracture group.

The faecal excretion of CaO remained fairly constant during the healing process, being slightly greater during the later periods. On balancing intake with output in this normally healing group, it is obvious that the data reveal positive and negative retentions both in the earlier and later periods. On the average these fluctuations tend to compensate.

Table I. *Daily mineral exchange (g.).*

Case	Period	Wt.	Age	Fracture	Time in days	In-take of N	Output of N Urine Faeces	Balance of N	In-take of CaO	Output of CaO Urine Faeces	Balance of CaO	In-take of P_2O_5	Output of P_2O_5 Urine Faeces	Balance of P_2O_5
1	A	65	21	Tibia	5-10	—	—	—	3.13	0.43	—	4.69	4.09	—
	B	65	21	Tibia	32-33	—	—	—	3.10	0.67	2.73	4.67	2.36	3.16
2	A	67	40	Femur	11-17	—	—	—	2.03	0.52	1.51	0.00	2.69	2.90
	B	67	40	Femur	42-47	—	—	—	2.06	0.59	1.68	0.21	3.16	2.06
3	A	60	32	Femur	6-13	10.63	15.77	2.20	2.12	0.77	1.79	0.44	3.47	3.87
	B	60	32	Femur	36-41	10.60	8.97	1.63	2.11	0.61	1.71	0.21	3.47	2.57
4	A	71	40	Femur	5-12	9.49	14.75	0.83	2.08	0.75	1.29	0.04	3.20	4.09
	B	71	40	Femur	40-45	9.49	9.52	1.27	2.08	0.65	1.46	0.03	3.20	2.04
5		67	34	T. & F.	4-10	—	—	—	3.25	0.47	2.69	0.09	4.59	3.46
6		71	62	Femur	56-63	13.28	12.74	0.43	2.19	0.57	1.34	0.28	3.86	2.505
7	A	60	19	Femur	6-12	—	—	—	2.05	0.47	1.06	0.52	2.61	3.60
	B	60	19	Radius	34-40	—	—	—	2.05	0.34	1.35	0.36	3.16	2.33
8		65	23	Femur & patella	8-16	—	—	—	2.11	0.48	1.71	0.08	2.97	3.72
9		40	19	Femur	7-14	—	—	—	2.96	0.74	2.23	0.01	4.46	3.61
10		65	31	T. & F.	6-13	—	—	—	1.39	0.65	0.74	0.00	2.29	3.10
11	A	69	29		13-20	—	—	—	3.13	0.91	2.67	0.45	5.26	3.88
	B	69	29		34-39	—	—	—	3.18	1.02	2.85	0.69	5.43	3.55
12		62	48	T. & F.	7-14	—	—	—	2.01	0.22	1.31	0.48	2.71	2.30
13		64	23	T. & F.	70-77	—	—	—	3.25	0.49	2.36	0.40	4.59	2.60
14		54	16	Femur	28-35	—	—	—	2.00	0.55	1.28	0.17	2.86	1.87
15		60	23	T. & F.	196-202	9.55	9.70	1.28	2.09	0.66	1.79	0.36	3.25	2.75
16	A	60	46	T. & F. & humerus	70-77	8.23	7.05	0.53	2.05	0.51	1.26	0.28	2.97	1.95
	B	60	46	T. & F. & humerus	105-112	—	—	—	2.07	0.49	1.12	0.46	3.05	1.76
17		64	33	Femur	42-49	—	—	—	2.15	0.70	1.485	0.035	3.75	2.295
19		68	40		6-11	10.33	12.60	1.52	2.19	0.50	2.07	0.38	3.47	2.79
20		60	18		5-12	9.98	13.65	1.75	2.10	0.43	1.70	0.03	3.26	2.77
21		41	14		5-12	8.75	9.45	—	2.05	0.43	—	—	—	—
22		55	21		3-18	—	—	—	1.97	0.38	—	4.88	3.28	—
23		67	40		—	13.29	12.64	1.28	1.92	0.37	1.65	0.10	3.76	2.66
24		68	19		—	13.51	13.23	1.16	1.21	0.39	0.81	0.01	—	1.18
25		68	40		—	13.28	12.86	1.17	1.96	0.385	1.54	0.035	3.79	2.53
26		71	62		—	—	—	—	1.54	0.14	—	4.26	2.81	—
27		60	25		—	—	—	—	1.47	0.25	—	4.44	3.00	—
28		70	40		—	—	—	—	1.18	0.22	0.91	0.05	—	—

"A" refers to early period. "B" refers to late period. Weights are expressed in kg. "Time in days" expresses time in days from date of accident and duration of the period during which excreta were collected. "T. & F." = tibia and fibula.

Table II. *Average daily mineral exchange of CaO and P₂O₅ in the various groups expressed as g. per kg. body-weight.*

Group	Period	Intake CaO	Output CaO		Balance CaO	Intake P ₂ O ₅	Output P ₂ O ₅		Balance P ₂ O ₅
			Urine	Faeces			Urine	Faeces	
A	Early	0.0355	0.0092	0.0257	+ 0.0006	0.0510	0.0554	0.0265	- 0.0309
	Late	0.0345	0.0087	0.0263	- 0.0005	0.0548	0.0354	0.0237	- 0.0043
B	Early	0.0375	0.0108	0.0287	- 0.0020	0.0584	0.0557	0.0297	- 0.0270
	Late*	0.0475	0.0152	0.0425	- 0.0102	0.0810	0.0529	0.0433	- 0.0152
E	Early	0.0384	0.0079	—	—	0.0646	0.0491	—	—
G		0.0230	0.0043	—	—	0.0613	0.0418	—	—

* Only one case.

Phosphorus. It is evident from Tables I and II that there is some gross disturbance of phosphorus metabolism, particularly during the earlier stages of the healing process. The average daily intake of P₂O₅ per kg. during the second week was 0.051 g. and during the second metabolic period 5-7 months later the intake was only slightly greater, 0.0548 g. During the earlier period the average urinary output of 0.0554 g. p.k.p.d. was greater even than the intake. During the later period the urinary output had declined to 0.0354 g. p.k.p.d. The loss to the body during these two periods was 0.0309 g. and 0.0043 g. p.k.p.d., that is, the loss was approximately seven times greater during the earlier period. The faecal excretion of P₂O₅ is also greater during the earlier period. The main excretory route for this excessive phosphorus loss is, however, the kidneys.

Nitrogen and sulphur. When the regular occurrence of this phosphorus loss was noted, the nitrogen balance was determined in a few of the remaining cases. In case 3 there was an average daily loss of 7.34 g. N during the second week. 5-6 weeks later the output balanced the intake. The P₂O₅ loss per day during the same periods was 2.27 g. and 0.29 g. respectively. The daily loss of N in case 4 was 6.09 g. during the second week and 1.30 g. during the seventh to eighth weeks. The P₂O₅ loss during these two periods was 2.32 and 0.14 g. respectively. Case 6 showed positive retentions of 0.11 g. N and 0.39 g. P₂O₅ per day during the eighth week. This subject had been massaged for a week previously.

Table III. *Case 29. The daily urinary excretion of an adult man with a fractured femur, dating from the second day following the accident.*

Daily excretion in g.					
Days	Urine		Days	Urine	
	Total N	Total P ₂ O ₅		Total N	Total P ₂ O ₅
2	7.92	1.91	8	19.9	3.65
3	24.9	3.10	9	16.7	3.16
4	32.5	3.88	10	16.7	3.16
5	25.2	4.75	11	14.0	2.73
6	22.7	4.75	12	15.1	2.78
7	21.0	3.33			

Daily intake of N = 14 g.

Table IV. *Case 30. This subject, a boy aged 15 years, was subjected to a double osteotomy of his femora for the correction of a deformity, on the third day of diet.*

Daily excretion in g.						
Urine						
Days	Total N	Urea-N	Ammonia-N	Total SO ₃	Inorganic SO ₃	Total P ₂ O ₅
1	10.08	7.96	0.437	1.965	—	2.46
2	10.08	8.14	0.430	1.86	—	2.60
3	6.72	4.93	0.277	1.39	—	2.26
4	13.96	11.53	0.781	2.50	1.77	2.88
5	12.39	10.06	0.437	1.99	1.69	2.54
6	11.76	9.35	0.596	2.00	—	2.68
7	12.73	8.60	0.437	1.935	—	2.07
8	10.30	8.26	0.555	2.08	—	2.10
9	12.39	9.01	0.403	2.15	—	2.245
10	12.49	—	—	—	—	—
11	12.18	—	—	—	—	—
12	11.23	—	—	—	—	—
13	11.44	—	—	—	—	—
14	11.13	—	—	—	—	—
15	11.55	—	—	—	—	—
16	11.23	—	—	—	—	—

Daily intake of N = 11.78 g. (except third day when diet was reduced).
This was the day of operation.

Table V. *Case 31. The daily urinary excretion of an adult man from the third day following a spiral fracture of the tibia caused by a fall of 3 ft. only.*

Days	Daily excretion in g.		
	Urine		
	Total N	Total SO ₃	Total P ₂ O ₅
3	12.2	2.07	2.41
4	14.1	2.57	3.09
5	14.5	2.79	3.24
6	17.4	2.83	3.64
7	16.1	3.10	3.46

Daily intake of N = 9.12 g.

Table VI. *Case 32. The daily urinary excretion of an adult woman from the second day following a fracture of the tibia.*

Daily excretion in g.			
Days	Urine Total N	Days	Urine Total N
2	9.24	7	13.38
3	11.865	8	9.03
4	10.99	9	9.87
5	12.18	10	9.36
6	13.28		

Daily intake N = 10.72 g.

Tables III, IV, V, VI and VII indicate the day-to-day changes in the urinary constituents following the fracture of bone. While the majority of the fractures were manipulated and set with the patient under the influence of an anaesthetic, case 33 who presented the greatest catabolism of the series had no such treatment.

Table VII. *Case 33. The daily excretion of a youth aged 16 years, from the third day following a fracture of both bones of one leg.*

Daily excretion in g.											
Days	Vol. of urine (cc.)	Total N		Total SO ₃		Total P ₂ O ₅		S : N ratio of excess output		P ₂ O ₅ : N ratio of excess output	
		Urine	Faeces	Urine	Faeces	Urine	Faeces				
3	630	12.02		2.61		3.48		1 : 14.44		1 : 5.96	
4	990	23.24		3.76		4.36					
5	1480	32.06		4.96		5.38					
6	1230	25.20		3.81		4.08					
7	1690	27.58		4.36		4.71		1 : 13.70	1 : 13.09	1 : 6.43	1 : 6.19
8	1680	24.01	Daily	3.98	Daily	4.11	Daily				
9	1390	19.25	av.	3.43	av.	3.78	av.				
10	1100	15.54	1.514	2.74	0.385	3.115	1.406				
11	1580	19.95		4.68		4.41		1 : 11.01		1 : 6.24	
12	1380	20.93		3.64		3.60					
13	1140	17.78		3.00		3.215					
14	1030	18.34		3.09		3.20					
Intake		11.70		1.931		3.560					

The S : N and P₂O₅ : N ratios of the excess outputs were determined in case 33. The figures in Table VII are the ratios of the loss taken in 4-day periods and also the total loss to the body during the period of observation—12 days.

B. Group of fracture cases treated with irradiated ergosterol (8–11).

Calcium. There was a tendency in this group for an increased urinary excretion of calcium to occur both in the earlier and later stages of repair. The mean urinary output of CaO during the second week was 0.0108 g. p.k.p.d., the intake being 0.0375 g. p.k.p.d. There was also a more general tendency for a negative retention to occur in this group. The active substance was administered in the form of "Radiostol" pellets (British Drug Houses, Ltd.). Each pellet is said to contain 6000 antirachitic rat units. Three of the cases received two pellets daily; case 10 received four.

Phosphorus. It was found in this group also that there was a distinct loss of phosphorus, particularly during the earlier period, the average daily loss being 0.027 g. p.k.p.d. Again the main excretory route for this catabolite was the kidneys.

C. Group of un-united fracture cases (12–15).

An attempt was made to determine if there were any gross deviations of the mineral metabolism in a group of un-united fractures. This group did not include such cases as are termed "pathological fracture," but consisted of a group which for unknown reasons had failed to heal.

The mineral metabolism of case 12 was examined during the second week following the re-fracture of a 9 months-old mal-united fracture. The patient had been in bed for a fortnight prior to the operation, and prior to that had moved about on crutches. The noticeable feature in this case was the absence of the marked phosphorus loss. Case 13 was a 10 weeks-old fracture of both

bones of the leg and case 15 was a compound fracture of both bones of the leg of 7 months' standing. This case showed slight negative retentions of calcium and phosphorus, and there was gross rarefaction of the bones visible by X-ray. At the operation performed 3 weeks later the friable nature of the bones was quite obvious. Case 14, a girl aged 16 years, fractured the neck of her femur by the simple process of knocking her toe against the pavement kerb. After 3 months there was still some degree of non-union, but eventually the bone healed. The X-ray evidence did not point to any bony abnormality.

D. Group of pathological fractures (16-18).

This group consists of three members. The first case (16) was a man who exhibited the triad of blue sclerotics, otosclerosis and bone fragility. He had suffered from four previous fractures, all of which had healed normally. His last injury (fifth) involved both bones of one leg. The tibia was fractured in two places, other cracks also being visible. The fibula was broken in one place. In addition, there was an impacted fracture of the surgical neck of the left humerus. These injuries were the result of a severe accident. Eleven weeks after the accident, although the humerus had appeared to unite well, there was still no union of the leg bones and no X-ray evidence of callus formation. Following the first experimental period, irradiated ergosterol was administered (three "Radiostol" pellets daily). A few weeks later definite callus formation was visible. A second metabolic period was instituted 1 month after the first. The urinary excretions in both periods were of the same order as were found in the normal fracture group. There was a small but definite retention of both calcium and phosphorus during the first period and this was increased considerably during the second, the CaO retention rising from 0.0047 g. to 0.0079 g. and the P_2O_5 from 0.0040 g. to 0.0070 g. p.k.p.d. Some degree of union took place, but the recovery was imperfect. The capacity for bone healing in osteogenesis imperfecta is supposed to be unimpaired and the unsatisfactory result in the above case is probably to be attributed to the badly fractured bone and not to any intrinsic cause.

The abnormality of the second case (17) was only discovered accidentally on X-raying the patient. The patient appeared to be in perfect health, but the X-ray plate showed marked fibro-cystic changes in the right femur and left ileum. There were slight changes also visible in the left femur and pelvis generally. No other evidence of bony changes was present. The Wassermann reaction was negative. The right femur which was fractured healed perfectly, the injured leg having been massaged from the third day onwards. The mineral metabolism showed no deviation from that of the normal fracture group. There was, however, a small retention of phosphorus. A similar retention was found in case 6 of the normal group. Both of these cases received massage. Work on the influence of massage is being delayed for a subsequent communication.

The third case (18) (Table VIII) of this series also showed fibro-cystic changes in the bones, particularly the pelvis. The cyst-like areas were numerous,

small and ill-defined in the pelvis, but more obvious in the femora. This patient had passed successfully through five pregnancies, but while out walking 7 weeks after the birth of the sixth child she felt a sudden pain over the symphysis pubis and had to be carried home. Double fractures of the rami of the os pubis and ischium were found. After $4\frac{1}{2}$ months in bed she was able to walk about 20 yards, her gait being more a waddle than a walk. This case had a defective dietary history. She did not eat butter, and milk was taken only in her tea. Whilst pregnant she usually took buttermilk and some butter. Metabolic periods were held 5, 7 and 19 months after the original fracture (*vide* Table VIII). Following on the first metabolic period, irradiated ergosterol was administered daily (four pellets of "Radiostol"). While there was initially a slight calcium retention and phosphorus loss (the patient having been in bed for some time previously), the administration of the active substance caused marked retentions of calcium and phosphorus which were maintained for over a year. At first the retention of phosphorus was relatively greater than that of calcium, but this was due, in part, to the fact that the patient had recovered sufficiently to be able to walk about more freely. The disused muscles were then able to recover their normal condition. This case ultimately made a very satisfactory recovery although she still has occasionally deep-seated pain. (A fuller clinical account of this case will be published elsewhere.)

E. Group of cases with injury to non-bony parts.

This group includes a case operated on for the removal of a piece of torn cartilage (19), a case of muscle laceration (20), a case of muscle incision (21), and a perforating spike wound of the leg (22).

Calcium. The urinary excretion of calcium is not much lower than that of the normal fracture group. There is also a tendency for a negative retention to occur.

Phosphorus. There is a marked loss of phosphorus. This loss is not quite so marked as that found to occur during the earlier days of the healing of a fracture. The kidneys again form the chief excretory route.

Nitrogen and sulphur. The daily average loss of nitrogen from the sixth till the eleventh days in case 19 was 3.79 g. per day, the P_2O_5 loss being 1.18 g. The average daily loss of nitrogen in case 20, from the fifth till the twelfth day, was 5.42 g., the P_2O_5 loss being 1.21 g. The $P_2O_5 : N$ ratios of these excess outputs are 1 : 3.2 and 1 : 4.7 respectively.

The daily urinary excretion of sulphur (SO_3), nitrogen and phosphorus (P_2O_5) of case 22 is represented in Table IX. This is a record from the second day following the accident.

F. Group of cases presenting abnormal mineral metabolism.

Brief mention only will be made of these cases and only in so far as they concern the present work.

It is hoped to publish fuller details of case 34 in the near future. This case when first seen at the age of 18.5 years had been unable to walk for 5 years

Table VIII. *Daily mineral exchange (g.).*

Case	Period of collection	Intake of CaO	Output of CaO		Balance of CaO	Intake of P ₂ O ₅	Output of P ₂ O ₅		Balance of P ₂ O ₅
		CaO	Urine	Faeces	CaO	P ₂ O ₅	Urine	Faeces	P ₂ O ₅
18	1. Ordinary diet	1.82	0.38	1.36	+ 0.08	2.75	1.66	1.17	- 0.08
	2. Ordinary diet								
	+ irradiated ergosterol (2 months' treatment)	1.95	0.29	1.15	+ 0.51	—	—	—	—
	3. Ordinary diet								
	+ irradiated ergosterol (17 months' treatment)	1.72	0.35	0.71	+ 0.66	2.48	1.34	0.57	+ 0.57
34	1. Ordinary diet	1.01	0.18	0.76	+ 0.067	1.61	0.64	0.97	0.00
	2. Ordinary diet	1.005				1.61			
	+ calcium glycerophosphate	1.02	0.16	1.58	+ 0.285	1.29	—	1.08	—
	3. Ordinary diet	1.06				1.77			
	+ calcium glycerophosphate	1.64	0.30	0.65	+ 1.75	2.08	1.48	0.57	+ 1.80
	+ irradiated ergosterol								
	4. Ordinary diet	1.06				1.77			
	+ phytin	1.12	0.26	0.89	+ 1.03	3.36	1.71	1.74	+ 1.68
	+ irradiated ergosterol								
	35 Ordinary diet	2.845	0.166	2.26	+ 0.42	4.35	1.79	2.35	+ 0.21

Table IX. *Case 22.*

Daily excretion in g.

Days	Urine					
	Vol.	Total N	Urea-N	Creatinine-N	Total SO ₃	Total P ₂ O ₅
2	960	17.85	14.70	0.79	2.88	2.63
3	880	18.27	15.39	0.84	3.18	0.17
4	1130	16.90	14.45	0.84	3.00	4.05
5	1090	14.49	—	0.615	2.64	4.10
6	1010	14.80	—	0.52	2.53	3.26
7	1080	13.44	—	0.58	2.30	3.08
8	1500	12.39	—	0.50	2.23	3.07
9	1270	11.71	—	0.72	2.03	2.86
10	—	11.55	—	0.63	2.09	2.97
11	1100	11.33	—	0.72	2.20	3.10
12	1150	10.29	—	0.58	2.08	2.47
13	1388	10.60	—	0.615	1.90	2.41
14	1490	11.34	—	0.56	1.94	2.84
15	1330	10.71	—	0.63	—	3.16

Daily intake of N = 14.35 g.

owing to weakness and pain of her thighs and legs. She had little, if any, interest in her food, and her intake of minerals had been particularly low. It was difficult to arrive at a diagnosis of her condition. The X-ray plates showed changes somewhat similar to those found in late rickets and osteomalacia but yet they were not typical. The administration of irradiated ergosterol (3-4 pellets "Radiostol" daily) caused very marked clinical improvement and by the end of 2½ months the patient was able to walk with considerable freedom. The inorganic phosphorus of the whole blood remained persistently low

(1.4–1.6 mg. per 100 cc.), but the calcium of the serum did not fall below 9.35 mg. per 100 cc. at any time.

A clinical description of the next case (35), one of osteitis deformans, has already been published [Cuthbertson, 1927] and the analytical data presented here are abstracted from that paper.

G. *Control group of healthy adults.*

Several of these cases had been in bed for varying periods with one leg fixed in a splint. The metabolism of case 23 was examined during a period of 2 days' activity followed by a week's rest in bed with one leg in a splint. Case 24 was examined during a period of 9 days' confinement in a splint, and case 25 during a period of 12 days' similar confinement. The data presented are the average daily metabolic exchanges during the particular period. These figures are abstracted from a previous communication on the effect of disuse on metabolism. Cases 26–28 were not confined to bed and led active lives.

DISCUSSION.

Before attempting to discuss the observed phenomena, it is first of all necessary to recall the chief events in the healing process. Without reference to any particular theory, it is held that about 4–6 days after an injury involving the fracture of bone, there are found areas of denser staining in the semi-elastic blood clot, which apparently represent the beginning of the deposition of calcium salts. In about 10 days, the early callus, a thick tenacious material, joins the bone ends and invades the adjacent muscle bundles. After about a month this callus has been absorbed or altered and there is in its place a firm mass which allows of only slight movement. Later this ill-defined callus assumes more the natural shape and consistency of the bones and union becomes more complete. Marked changes occur at the ends of the fractured bones. There is an apparent absorption of both matrix and cells—a process of demineralisation and atrophy. Leriche and Poligard [1928] believe that the genesis of new bone trabeculae is conditioned by the absorption of the pre-existing bone, and that this process of rarefaction begins immediately after the trauma. There is thus a process of bone absorption coincident with an anabolic phase. Where apposition is poor the bones may waste themselves on an impossible task. In case 11 the apposition appeared to be fairly good, and there was a definite formation of callus visible on the X-ray plate; yet the bones had not united, although there was no apparent cause. Gross rarefaction, particularly of the proximal fragment of the femur, was clearly visible and union ultimately took place after the two fragments were plated. Such a wasting was also visible in the X-ray plates of case 15. At the operation performed 7 months after the injury there was no cause for non-union, other than the separation of the fragments. Very little callus had been laid down and the bones were soft. The bone ends were freshened by shaving the apposed surfaces, and the bones plated. The bone was so soft that the nails held their position with great difficulty.

Union followed. A fragment from the end of the fibula was analysed and it was found to have a $\text{CaO} : \text{P}_2\text{O}_5$ ratio of 1.43 : 1. The normal ratio for bone is 1.47 : 1 assuming the percentage of CaO and P_2O_5 to be 28.85 % and 19.55 % respectively [McCrudden, 1910]. A piece of callus removed at open operation on a case of badly apposed bone fragments showed a ratio of 2.3 : 1. This would appear to support the view put forward by Schwarz, Eden and Herrmann [1924] that in ossification calcium is first laid down in organic combination, and that subsequently union with phosphorus occurs.

From the available data presented in this report the following observations can be made.

Calcium. The metabolism of calcium as determined by the intake and output of CaO during the different stages of the healing process in fracture cases shows but little deviation from the normal. Although calcium may sometimes be retained in small amount there is as often a tendency to a slight but continuous loss. It is difficult to trace any relationship between the positive and negative retentions and the phase of healing.

It has long been known that certain substances, *e.g.* milk, various calcium salts, etc., can increase the urinary output of CaO . Table X illustrates the effect of the administration of 10 g. calcium glycerophosphate on two consecutive days to a patient with a fractured spine, who had been in bed for a month previously. The increased excretion was however slight. Curiously enough, there was a fall in the urinary excretion of phosphorus.

Table X. *Case 35. Urine.*

Days	Urine			
	Intake CaO (g.)	Output CaO (g.)	Intake P_2O_5 (g.)	Output P_2O_5 (g.)
1	1.065	0.476	4.54	3.31
2	1.065	0.483	4.54	3.18
3	1.065	0.476	4.54	3.57
4	1.065	0.497	4.54	3.32
5	1.065	0.465	4.54	3.35
6	1.065	0.511	4.54	3.50
7	1.065	0.476	4.54	3.50
8	1.065 + 1.224	0.546	4.54 + 1.556	2.73
9	1.065 + 1.224	0.586	4.54 + 1.556	3.07
10	1.065	0.553	4.54	3.82
11	1.065	0.533	4.54	3.70
12	1.065	0.513	4.54	3.62

While it has been suggested that the kidney supplies the sole, or at any rate the main, route for the excretion of calcium [Telfer, 1922], the recent work of Grosser [1920], Husband, Godden and Richards [1923], Bergeim [1926], and Stewart and Percival [1927] has indicated that the main excretory route is probably through the wall of the large intestine, and that the urinary calcium represents but a small fraction of the total excretion.

The urinary excretion of CaO cannot be taken as an indicator of the phase of calcium metabolism, for an increase may be found when bone is rarefying and when it is being laid down. In osteomalacia, it is said that the amount of calcium passed in the urine may be so great that it is visible to the naked eye as renal

sand. In case 34 there was an increased urinary excretion only when there was a coincident increased retention. Mere increasing of the intake of lime was ineffective in this case without the addition of irradiated ergosterol. Further, a decrease of the urinary excretion of calcium may be found either when bone is being laid down in excess as in certain phases of osteitis deformans (case 35), or in rickets where there is a primary or secondary diminished absorption.

The average urinary calcium excretion of the fracture cases treated with irradiated ergosterol was 0.0108 g. p.k.p.d. This increase which results from the administration of the irradiated substance is not much greater than the value found in the untreated group. The ergosterol may merely accelerate the normal process. Harris [1930] quotes the unpublished observations of Watchorn to the effect that this irradiated substance causes a marked increase in the urinary excretion of calcium.

This increased urinary calcium excretion in the fracture cases is not coincident with an increased retention of calcium or phosphorus, for there is often a slight loss. This finding coupled with the effect of irradiated ergosterol on the metabolism of case 34 would appear to furnish some evidence in support of the theory that the primary effect of this vitamin is on the absorption of mineral matter from the gut, rather than a direct stimulation of the tissues to calcify. This increased absorption may result irrespective of any increased demand from the skeleton. On the other hand, it may be that this active agent has some effect on the intermediary metabolism, as has been suggested by Hess [1929].

In a fracture the whole healing process may be purely local at first, the mineral elements coming from the adjacent bone fragments, and only subsequently is there an actual retention of minerals to make good the depleted bone. There is probably in addition a slight loss due to disuse of the body generally which may be masked by an increased deposition at the site of fracture. Another possible explanation would demand that in addition to an active demineralisation resulting in a loss of mineral salts, there is active deposition in the junctional area, the two processes not however being in the nature of a single reversible reaction, but separate, the deposited substance not necessarily coming from the atrophied bone. On the available evidence it is impossible to decide which is the natural method. The latter would be a distinct disadvantage to a wounded animal cut off from its food supplies, and would not be in the interests of natural economy.

Phosphorus, nitrogen and sulphur.

While the metabolic exchange of calcium shows only a slight deviation from the normal, that of phosphorus is very definitely altered. There is an excessive loss of this element during the earlier phases of the healing process. By the end of 1-2 months there is only a slight loss. The main excretory route for this excess output is by the kidneys. During the second week of healing there is, on the average, seven times as much phosphorus being lost as during the fifth to eighth weeks.

It is obvious that bone cannot be the main source of this phosphorus, for the loss is too early and too great, and further it is coupled with an excessive loss of nitrogen and sulphur. The main source of these catabolites cannot be directly from the absorption and the elimination of the effused blood at the site of fracture, for the loss is too great.

There remains for consideration the muscular system. The question naturally arises as to what disturbance of muscular metabolism would lead to such an enormous and early loss of substance. Tables III and VII illustrate the rates of excretion of nitrogen, phosphorus and sulphur following the fracture of bone. These cases presented the greatest catabolism of the series. They demonstrate how early this loss reaches its maximum. On the other hand Tables IV, V and VI present excretory rates which are not of a much greater magnitude than are found in cases of pure disuse due to immobilisation in splints. The S : N and P_2O_5 : N ratios of the total excess output in case 33 were 1 : 13.09 and 1 : 6.19 respectively during the period of observation. It will be noted that during the later days there is a relatively greater output of sulphur in relation to the loss of nitrogen. The phosphorus output, on the other hand, maintained a more constant relationship to the nitrogen. The generally accepted S : N and P_2O_5 : N ratios for muscle are 1 : 14 and 1 : 6.6 respectively. Although due consideration must be allowed for the shortness of the period of observation the ratios found in case 33 suggest that the main source of the substance catabolised is some sulphur-rich complex store material, which is probably intimately connected with muscle.

It has long been observed that disuse of muscle leads to atrophy, and the natural question arises as to the rôle played by pure disuse in the disturbed metabolism of fracture cases. This question has, in part, been already investigated by the present writer [Cuthbertson, 1929], who found that normal subjects in nitrogenous equilibrium showed within a day or two from the commencement of a period of muscle rest involving fixation of a lower limb in a splint coupled with rest in bed, a rise in the excretion of sulphur, nitrogen, phosphorus and later calcium in that order of priority. This rise was maintained fairly steadily for a varying period after which it gradually declined. The S : N ratio suggested a sulphur-rich source for the excreted material which was presumably muscle. While the loss of sulphur, nitrogen, and phosphorus during such an experimental period of imposed rest, though considerable, does not reach such a high value as is found following a fracture of bone there appears to be some qualitative resemblance. That the degree of immobilisation is considerably more absolute in the case of a fracture and may lead to a much earlier and greater loss of muscle substance is very probable, but this explanation does not appear to be sufficient adequately to account for the loss found to occur in wounds of non-bony parts, where there has been little, if any, fixation. These changes found in cases 19–22 which involve a loss of sulphur, nitrogen and phosphorus were similar to those produced by a fracture, but of less extent. Many years ago Bauer [1872] noted that an increased nitrogen

elimination in the urine is found after blood-letting. The experiments of Turner, Marshall and Lamson [1915] and Taylor and Lewis [1915] illustrate the rapidity of the renewal of the blood constituents. Their experiments indicate an increased metabolism of protein.

Apart from pure disuse there remain two other possible explanations to be considered. It has long been known that muscular wasting associated with bone or joint disease is much more rapid and extreme than that which occurs with simple disuse of a limb. It is also true that there is a more rapid and extreme wasting in limbs immobilised for some inflammatory or traumatic lesion than in limbs immobilised to correct some deformity. The reason for this lies not so much in the degree of immobilisation, but in some reflex trophic effect. Harding [1925, 1926, 1929] extending the observations of Raymond [1890], Hoffa [1890], and Langley *et al.* [1915, 1917] has shown that such an atrophy, which is mainly reflex in character, depends on the integrity of the afferent paths. This theory demands an exhaustion of the muscle by an excessive number of stimuli. The present writer [Cuthbertson, 1929] found that, following a period of simple disuse in a man whose metabolism was normal, operative removal of a fragment of semi-lunar cartilage under spinal anaesthesia resulted in a few days in a very rapidly increasing loss of substance, which was presumably muscle. This excessive catabolism only commenced to fall after the patient had been out of bed for a day and a half. During this post-operative period the patient's legs were only loosely held between sand-bags. It is difficult to suggest any other explanation than that of a reflex trophic effect to account for this considerable loss of substance, as the increased catabolism was far in excess of that produced in an earlier period of experimental immobilisation. May then this increased catabolism found in fracture cases be mainly reflex in origin also?

One other possible contributory condition remains to be examined, namely, toxic waste. Here our knowledge is extremely vague. When tissue is broken down within the body, there are eliminated a great many cellular products whose final elimination is due to an enzymic dissolution. Just as histamine and allied compounds appear to play such a large part in the production of shock, so some allied compounds formed from the breaking-up of the cellular debris in the fractured or injured area may cause a disturbance of the normal balance of the body cells resulting in an imbalance between anabolism and catabolism. A small febrile rise is occasionally found in cases of injury, but it is seldom pronounced if the fracture be simple, and lasts at the most but a day or two. In cases 29 and 33 where the greatest catabolisms were observed, no rise was noted in case 29 and only a rise of 0.5° F. in case 33.

In very general terms the gross disorder of metabolism in fracture cases may be taken to be the result of two effects—disuse atrophy and tissue injury.

Unfortunately this investigation throws little or no light on some of the unknown causes of non-union.

The exhibition of irradiated ergosterol did not appear to facilitate the

healing process in what was presumed to be a normal group of fractures: indeed the clinical after-history was one of delayed union. The group is admittedly too small on which to base any general clinical deductions, but since its members were selected at random it would suggest that this form of therapy should not be indiscriminately used in normal cases without more experimental evidence. Certain cases of pathological fracture and abnormal bone metabolism appear, however, to benefit very markedly from the addition of this vitamin to their diet.

Table XI. *Blood analyses.*

Case	Age	Injury or fracture	mg. per 100 cc.				
			Time in days between injury and operation	Inorganic P		Nitrogen	
				Plasma	Whole blood	Non- protein	Urea
30	14	Double osteotomy of femora	—	—	4.5	36	20
			4	—	4.1	29	17
36	39	Humerus	4	—	2.1	29	17
37	35	Tibia	4	3.2	—	35	13
			11	2.5	—	—	—
38	36	Tibia	4	1.5	—	36	—
			10	1.4	—	—	—
39	42	Femur (compound)	21	3.1	—	—	—
40	54	Femur	5	2.8	—	—	—
			21	2.1	—	39	—
41	50	Femur	28	4.2	—	—	—
42	19	T. & F.	3	3.3	—	—	—
			11	4.5	—	—	—
43	21	T. & F.	3	3.9	—	—	—
			11	5.0	—	—	—
44	33	Femur	20	5.3	—	—	—
			29	4.9	4.2	32	—
45	19	Tibiae	60	3.4	—	—	—
46	20	Laceration of leg	7	3.5	—	—	—
			20	3.3	—	44	—
47	17	T. & F.	8	5.0	4.8	30	—
48	30	T. & F.	24	—	1.5	—	—
49	50	T. & F. (compound)	7	3.8	—	39	—
			14	—	3.5	—	—
50	43	T. & F.	21	3.9	—	—	—
51	23	Tibia	7	3.6	—	39	—
			14	—	3.7	60	—
52	22	T. & F. (compound)	21	4.1	4.2	—	—
			28	—	4.2	34	—
53	66	T. & F.	21	—	3.3	—	—
			28	—	3.6	—	—
54	31	Femur	4	3.2	—	31	—
			14	4.4	—	44	—
55	23	T. & F.	3	3.6	—	—	—
			14	3.8	—	41	—
56	23	Tibia	7	4.0	—	36	—
57	37	Laceration of leg	7	2.4	—	32	—

T. & F. = tibia and fibula.

Blood analyses.

As was suggested earlier in this paper, the work of the various investigators on the inorganic phosphate of the blood in fracture cases indicates that there probably is a rise in the level of this element during the earlier weeks of healing. The evidence also suggested that such a rise was not specific for wounds of bone, but might follow non-bony injury. When it became evident that there was an early and very considerable loss to the body of sulphur, nitrogen and phosphorus, the question naturally arose as to whether the rise in the blood-phosphorus might also be an indicator of the increased catabolism. If such were the case then one might also expect to find an increase in the inorganic sulphur and non-protein nitrogen in the blood. The present series of blood analyses are concerned with this question. They were performed at varying intervals from the date of injury, and with few exceptions all were from adults. The patients had fasted for 12 hours previously.

It is generally held that there is an approximately even distribution of inorganic phosphorus between corpuscles and plasma, the upper limit of normality being 3.5 mg. per 100 cc. The data indicate that in many cases there is a rise in the inorganic phosphorus to over 3.5 mg., but that healing may occur without such a rise. This rise is not contemporaneous with the maximum catabolism noted in the urine, but occurs during the second and third weeks, declining to the normal later. There appears to be no coincident rise in the non-protein-nitrogen or urea of the blood although subsequently there is a tendency for the values to approach the upper limits of normality. Further work is being done to determine the significance of the changes which occur during the later weeks, for they seem to be intimately connected with the process of repair, but whether of bone or soft tissue or more probably both it is at present impossible to say.

CONCLUSIONS.

In conclusion it seems reasonable to seek an interpretation for the biological significance of these phenomena associated with injury. Why is there such an early and rapid catabolism of the tissues? Further, is there any significance to be attached to the fact that the evidence of maximum catabolism is contemporaneous with the first signs of regeneration of the injured part?

Injury to an animal generally leads to lessened activity and a lessened activity usually results in a diminished food supply. The necessity is urgent, and the body may require to catabolise its reserves to meet both the exigencies of repair and of maintenance.

In simple disuse the catabolic disturbance, although not of the extent sometimes found associated with injury, appears to be qualitatively the same. Similarity of response, however, must not be taken as an indication that the primary causes are identical. Simple disuse, that is disuse not caused by any

abnormal condition of the tissues, is largely an experimental state, and is seldom found in nature apart from injury. As disuse leads to decreased activity, it is again essential for the animal to economise. The non-functioning part may require to be sacrificed for the whole.

It is possible that some such hypothesis of urgency may account for the relatively increased protein catabolism found to occur during the second to fourth days in the majority of starved subjects.[Luciani, 1890; Munk, 1893; Johansson *et al.* 1897; Benedict, 1907, 1915; Cathcart, 1907]. The general assumption is that the protein reserves are being catabolised in an endeavour to maintain a constant level of metabolism following on the earlier and more rapid exhaustion of the carbohydrate reserves, and that any slight increase in heat production is due to the specific dynamic action of the protein. Such may indeed be the true interpretation of the data, but in addition they seem to suggest that this temporary and early acceleration of the catabolism of protein and also probably fat is a special effort to meet an urgent need.

SUMMARY.

1. In normally healing fracture cases the metabolism of calcium showed but little change. Intake on the average balanced output. There was, however, a marked loss of nitrogen, phosphorus and sulphur, the main excretory path for these catabolites being the kidneys. The rate of loss following the injury reached a maximum about the second to sixth day and then gradually declined. There was still a slight but continuous loss even after the lapse of 1 or 2 months.

2. The S : N and P_2O_5 : N ratios suggested that the material catabolised was probably mainly muscle.

3. In otherwise normal fracture patients, the administration of irradiated ergosterol did not appear to alter the metabolism apart from a slight increase in the urinary excretion of calcium; nor was there any special clinical improvement to be attributed to this form of therapy.

4. Metabolic analyses did not throw any light on the problem of the un-united fracture.

5. The beneficial effects of irradiated ergosterol in certain types of pathological fracture and abnormal bone conditions have been demonstrated.

6. A catabolic loss of sulphur, nitrogen and phosphorus resulting from the injury of tissue other than bone has been demonstrated.

7. Brief mention has been made of a few cases of abnormal bone metabolism in so far as they illustrate some of the phases of calcium and phosphorus metabolism.

8. There does not appear to be any coincident increase in the non-protein-nitrogen and inorganic phosphorus of the blood corresponding to the increased catabolism noted in cases of fracture and non-bony injury. Changes do occur later, which appear to involve a raising of the level of these elements.

I wish to thank Prof. E. P. Cathcart for his encouragement and helpful criticism. My thanks are also due to Prof. P. Patterson, Messrs Patrick, Macewan, Milne McIntyre, Duff, Taylor, Stevenson and Dr A. Hunter for their active co-operation and help in supplying me with cases.

I am indebted to British Drug Houses, Ltd., for a supply of "Radiostol" and to the Clayton Aniline Co., Ltd., for a supply of phytin.

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