STUDIES ON EXPERIMENTALLY PRODUCED FRACTURE IN DOGS: PART II—CHANGES IN TOTAL, NON-PROTEIN AND PROTEIN NITROGEN CONTENT.

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Cuthbertson (1930, 1931, 1932 and 1936) has shown that a severe injury, such as the fracture of a long bone, leads to increased elimination of urea and other nitrogenous substances in the urine. The nitrogen excretion may exceed the nitrogen intake and remain at a higher level for several weeks after the injury if the patient is kept on an ordinary diet (Howard *et al.*, 1944). The consequent nitrogen depletion cannot be countered by feeding protein rich diet (Cuthbertson, 1932 and 1936). Cuthbertson *et al.*(1939) and Munro and Cumming (1948) were of the view that the greater part of the nitrogen lost from the body, after fracture, is drawn from sources other than the injured limb. Several other workers have also reported abnormal losses of body nitrogen after bone fracture (Munro and Chalmers, 1945; Sacher *et al.*, 1950). Ingle *et al.* (1947) have shown that urinary excretion of non-protein nitrogen increases both in human patients and in laboratory animals following fracture and other types of stress.

Although considerable literature has accumulated on the loss of nitrogenous substances after bone fracture, no work appears to have been done on the distribution of these substances at the focus of injury. In the present sets of experiments an attempt has been made to investigate the alterations in the concentration of total, non-protein and protein nitrogen in the circulation as also at the site of the experimental fracture during the earlier stages of fracture healing.

EXPERIMENTAL PROCEDURE

Male mongrel dogs (11.7-15.4 kg. body weight) were used as the experimental animals in these studies. Under ether anaesthesia and with aseptic precautions, fracture was produced by open operation on the middle third of the shaft of right femur (Ghatak *et al.* 1957).

Method of Estimation

Total Nitrogen : 0.25 cc of blood serum or exudate was digested with 0.5 cc concentrated sulphuric acid and two drops of hydrogen peroxide in a

microkjeldahl flask for about one hour and the total nitrogen (TN) content was determined by direct nesslerisation procedure. The colour intensity was read in a Klett–Summerson photoelectric colorimeter at 420 mμ.

Non-protein nitrogen : 1.5 cc of 10 per cent trichloracetic acid was added to 0.5 cc of serum or exudate to precipitate the proteins, which were removed by centrifugation. One cc. aliquot of the protein-free filtrate was digested with sulphuric acid and hydrogen peroxide and non-protein nitrogen (NPN) content was determined by direct nesslerisation.

Values for protein nitrogen (PrN) were calculated by subtracting the NPN values from the total nitrogen.

The data obtained were analysed statistically.

RESULTS

Effect of ether anaesthesia on total, non-protein and protein nitrogen content.

Blood was withdrawn from the dogs, kept under standardised dietary conditions, before and after administering ether anaesthesia, and total nitrogen and non-protein nitrogen contents determined in the serum. Statistical analysis of the data given in Table I indicates that the differences from the normal (before anaesthesia) are not significant at 5 per cent level.

TABLE I

Statistical analysis of the data on the effect of ether anaesthesia on the total, non-protein and protein nitrogen contents of blood serum

	Difference in (after anaesthesia-normal)		
	Total nitrogen*	Non-protein Nitrogen**	Protein nitrogen**
Mean difference	—0.0133	+0.0017	—0.0067
S. E. of mean difference	0.0110	0.0128	0.0456
$\frac{t}{P}$	—1.209	+0.133	—0.147
	>0.20	>0.90	>0.80

* Observations of 8 dogs

** Observations of 7 dogs

Effect of fracture of the femur on the total, non-protein and protein nitrogen contents :

Blood :—Blood samples were withdrawn ½, 24, 48, 72, 96 and 120 hours after fracture and the different values obtained are recorded in Table II.

TABLE II

Effect of fracture on total, non-protein and protein nitrogen contents of blood serum (mg./ml.)

S. N. of animal & weight (kg)	Estimations	Normal (before anaesthesia)	After anaesthesia	Interval after fracture (hours)					
				½	24	48	72	96	120
1	Total N	12.75	12.62	13.30	11.97	11.46	11.00	10.70	10.65
13.5	N. P. N.	1.23	1.21	1.22	1.21	1.19	1.19	1.16	1.15
	Pr. N.	11.52	11.41	12.08	10.76	10.27	9.81	9.54	9.50
2	Total N	10.75	10.64	11.04	11.30	10.00	9.70	9.40	9.30
12.3	N. P. N.	1.07	1.04	1.06	1.05	1.04	1.04	1.03	1.04
	Pr. N.	9.68	9.60	9.98	10.25	8.96	8.66	8.37	8.26
3	Total N	12.70	12.70	12.95	13.10	11.40	11.10	10.60	10.60
13.4	N. P. N.	1.22	1.20	1.21	1.20	1.18	1.16	1.16	1.16
	Pr. N.	11.48	11.50	11.74	11.90	10.22	9.94	9.44	9.44
4	Total N	11.10	11.30	11.60	11.00	10.60	10.40	10.00	9.95
14.4	N. P. N.	1.08	1.10	1.12	1.06	1.06	1.04	1.04	1.05
	Pr. N.	10.02	10.20	10.48	9.94	9.54	9.34	8.96	8.90
5	Total N	11.00	11.00	11.30	10.75	10.38	10.25	9.50	9.55
12.0	N. P. N.	1.11	1.12	1.11	1.10	1.08	1.08	1.06	1.06
	Pr. N.	9.89	9.88	10.19	9.65	9.30	9.17	8.44	8.49
6	Total N	11.50	11.50	11.75	11.00	10.50	10.25	11.25	11.20
15.4	N. P. N.	1.05	1.11	1.05	1.05	1.05	1.05	1.00	1.05
	Pr. N.	10.45	10.39	10.70	9.95	9.45	9.20	10.25	10.15
7	Total N	10.20	10.20	10.60	10.10	9.80	9.60	9.40	9.30
11.5	N. P. N.	1.08	1.08	1.05	1.04	1.04	1.03	1.03	1.03
	Pr. N.	9.12	9.12	9.55	9.06	8.76	8.57	8.37	8.27
8	Total N	11.26	11.17	11.70	10.80	10.30	10.20	9.80	9.70
11.7									

It would be seen that practically in all the dogs the total nitrogen and protein nitrogen contents of the serum rises above the normal level soon after the fracture. This is followed by a progressive fall which reaches its minimum 96 hours after the fracture, the time at which usually the callus tissue starts forming.

The non-protein nitrogen level declines very slightly, though the fall is statistically significant.

Statistical analysis of the data (cf. Table III) shows that the differences in the total, non-protein and protein nitrogen concentration of the blood before and three days after fracture are significant at 5 per cent level. The same holds good with regard to the differences in the respective value after anaesthesia and three days after the fracture.

TABLE III

Statistical analysis of the data on the effect of fracture on total, non-protein and protein nitrogen contents of blood serum.

	Difference in(After 3 days of fracture-normal)			Difference in (After 3 days of fracture-anaesthesia)		
	Total N	N.P.N.	Pr. N.	Total N	N.P.N.	Pr. N.
mean difference	—1.052	—0.033	—1.150	—1.039	—0.035	—1.143
S. E. of mean difference	0.138	0.010	0.171	0.124	0.007	0.149
t	—7.647	—3.469	—6.729	—8.358	—4.861	—7.678
P	<0.001	<0.02	<0.01	<0.001	<0.01	<0.001

The differences in means are significant at 5 per cent level of significance.

Exudate :—The fluid collected at the site of fracture was aseptically aspirated ½, 24, 48, 72, 96 and 120 hours after the fracture. It was centrifuged and the estimations were made in the clear supernatant. The results presented in Table IV would show that soon after the fracture the total nitrogen and protein nitrogen contents of the exudate are much higher than the corresponding values in blood. The values drop sharply 24 hours after the fracture. The total nitrogen and the protein nitrogen contents of the exudate in some dogs remain throughout at levels higher than those in blood while in dogsNos. 3, 4, 6 and 7 the values fall below the corresponding values in blood 48 to 72 hours after the fracture.

Soon after the fracture, the non-protein nitrogen in the exudate has a tendency to rise above the corresponding value in the blood and keep at a high level throughout the period of experiment.

TABLE IV

Effect of fracture on total, non-protein and protein nitrogen content of exudate (mg/ml).

S. N. of animal & weight (kg)	Estimations	Interval after fracture (hours)					
		½ (Initial)	24	48	72	96	120
1	Total N	17.00	14.10	13.00	12.47	12.10	12.00
13.5	N. P. N.	1.75	1.40	1.21	1.21	1.15	1.16
	Pr. N.	15.25	12.70	11.79	11.26	10.95	10.84
2	Total N	13.60	12.00	11.50	10.10	9.50	9.35
12.3	N. P. N.	1.55	1.30	1.25	1.10	1.10	1.10
	Pr. N.	12.05	10.70	10.25	9.00	8.40	8.25
3	Total N	14.20	13.80	11.80	10.90	10.60	10.60
13.4	N. P. N.	1.70	1.45	1.40	1.20	1.30	1.25
	Pr. N.	12.50	12.35	10.40	9.70	9.30	9.35
4	Total N	14.10	13.20	10.20	10.00	9.80	8.90
14.4	N. P. N.	11.62	1.41	1.36	1.28	1.20	1.20
	Pr. N.	12.48	11.79	8.84	8.72	8.60	8.70
5	Total N	13.25	10.00	9.00	8.00	9.00	8.80
12.0	N. P. N.	1.60	1.35	1.25	1.15	1.15	1.15
	Pr. N.	11.65	8.65	7.75	6.85	7.85	7.65
6	Total N	13.75	13.50	12.50	9.75	10.50	10.65
15.4	N. P. N.	1.65	1.35	1.28	1.05	1.05	1.10
	Pr. N.	12.10	12.15	11.22	8.70	9.45	9.50
7	Total N	12.10	10.70	9.70	8.60	8.30	8.20
11.5	N. P. N.	1.50	1.35	1.15	1.10	1.05	1.05
	Pr. N.	10.60	9.35	8.55	7.50	7.25	7.15
8	Total N	14.5	9.8	9.2	9.0	8.8	8.6
11.7							

Statistical analysis of the data (cf. Table V) would indicate that the fall in the values of total, non-protein and protein nitrogen three days after the fracture, as compared with the figures just after fracture, is significant at 5 per-cent level.

TABLE V

Statistical analysis of the data on the effect of fracture on total, non-protein and protein nitrogen contents of exudate.

	Difference in (after 3 days-just after fracture)		
	Total nitrogen	Non-protein nitrogen	Protein nitrogen
mean difference	—4.3644	—0.4800	—3.9167
S. E. of mean difference	0.3443	0.364	0.4552
t	—12.676	—13.187	—8.604
P	<0.001	<0.001	<0.001

The differences are significant at 5 per cent level

Ratio of total nitrogen to protein nitrogen

The crude data have further been statistically analysed to see if there occurred any change in the total nitrogen to protein nitrogen ratio as a result of anaesthesia as well as fracture in blood and the exudate.

TABLE VI

Ratio of total nitrogen to protein nitrogen in venous blood and in the exudate

	Blood			Exudate	
	Normal	after anaes-thesia	after 3 days of fracture	½ hour after fracture (INITIAL)	after three days of fracture
Ratio of TN/PrN	1.1072	1.1075	1.1169	1.1280	1.1289
± S. E. (Ratio)	$0.0^{3}6705$	$0.0^{3}5350$	$0.0^{3}4326$	$0.0^{2}3348$	$0.0^{2}2599$

Ratio do not differ significantly

It would appear from the results presented in Table VI that even though the concentrations of total, non-protein and protein nitrogen in blood and exudate differ significantly from the initial value three days after the fracture; the ratios of total nitrogen to protein nitrogen remain more or less the same.

DISCUSSION

The results presented above would show that the concentrations of total, non-protein and protein nitrogen are altered in blood and exudate as a result of the fracture of the middle third of the femur bone. The increase in the total and protein nitrogen contents of the blood noted half an hour after the fracture in most of the experimental dogs would indicate that there is a flow of nitrogenous compounds from the tissues into the circulation to meet the increased demand for these substances at the site of experimental fracture. In certain cases (dogs No. 2 and 3), the rise reaches its peak 24 hours after fracture. This initial rise is followed by a progressive fall to a constant level 96 hours after the fracture. The non-protein nitrogen level shows very slight decrease. It may be pointed out that although the concentrations of total, protein and non-protein nitrogen in blood are altered three days after the fracture, the ratio of total nitrogen to protein nitrogen remains the same.

In the exudate, however, the total and protein nitrogen rise much above the corresponding values in the serum, registering about 26 to 28 per cent increase soon after the fracture in dog No. 1. The values decrease later reaching a constant level 96 hours after the fracture. The ratio of total to protein nitrogen, however, is not altered. The non-protein nitrogen level of the exudate shows a similar trend. In some dogs the levels of protein and total nitrogen in the exudate were always higher than those in blood while in dogs No. 3, 4, 6 and 7 the values fell below the normal level. Considering the fact that there is an increase in the volume of the exudate with the lapse of time, the total quantities of the different nitrogenous substances present at the site of fracture would be much higher than those reported here. The increase in the concentration of these substances at the focus of injury would suggest that the nitrogenous substances also play an important role along with vitamin C in the repair process which set in after the formation of the callus tissue.

It is reasonable to assume that after utilization, the non-essential nitrogenous substances are discarded and excreted in the urine. This may explain the increased nitrogen output which occurs after fracture.

SUMMARY

1. Ether anaesthesia has no effect on the total, non-protein and protein nitrogen contents of blood.

2. The total nitrogen and protein nitrogen contents of the serum rise above the normal level soon after femur fracture. This is followed by a progressive fall which reaches a steady state 96 hours after the fracture.

3. The non-protein nitrogen level of the serum declines very slightly.

4. The total and protein nitrogen contents of the exudate at half hour interval are much higher than those in the blood. The values then drop reaching a constant level 96 hours after fracture.

5. The non-protein nitrogen in the exudate soon after fracture has a tendency to rise above the corresponding value in blood.

6. The ratios of total to protein nitrogen are not altered in the serum and the exudate as a result of fracture.

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