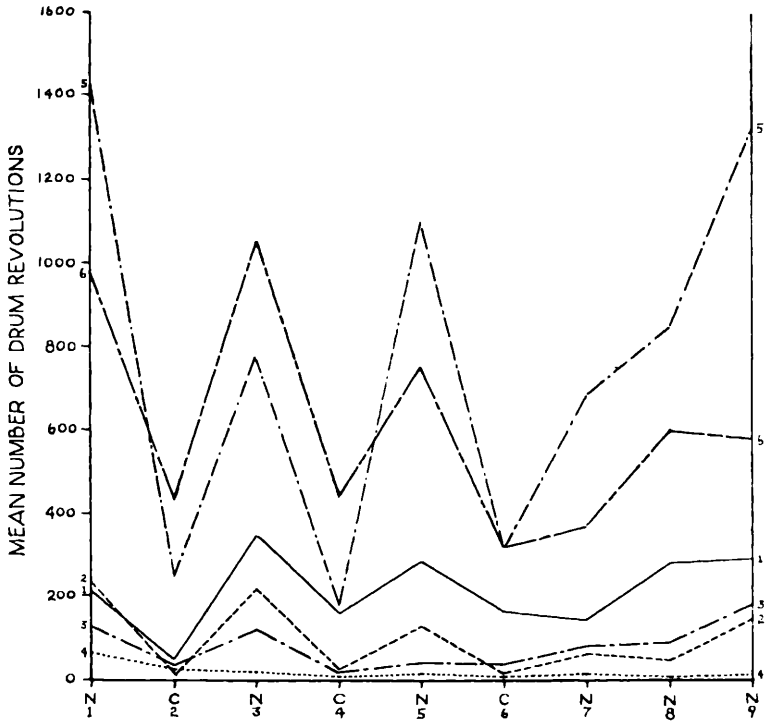

Fig. 1.

Activity records of 6 female rats. In periods 1, 3, 5, 7, 8, and 9 the usual conditions for rats in activity drums prevailed; whereas, in periods 2, 4, and 6 the rats received one electro-convulsive shock each day. Each rat's number is given on the left and right ordinate.

sive shock. Eventually, however, a disturbance of estrus might have been a contributing factor. Further studies on this and other aspects of the problem are in progress.

Conclusion. Electro-convulsive shocks in 6 female rats greatly reduced the amount of their voluntary activity for a short period of time. The major change appears within the first 24 hours and disappears within 48 hours after the last convulsive shock is administered. Within a 2-week period following their last convulsion the majority of the animals closely approximated their pre-shock levels of daily activity.

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Changes in the Hydrogen Ion Concentration of Healing Fractures.

ORVAR SWENSON AND C. LLOYD CLAFF. (Introduced by E. C. Cutler.)

From the Laboratory for Surgical Research, Harvard Medical School, Boston, Mass.

The repair process involved in fracture healing is of great interest both from theoretical and practical standpoints. Repair of bone differs from soft tissue repair, in that the process involves calcification and ossification. The morphological changes in fracture repair are well established and have recently been reviewed by Urist and Mc-

TABLE I.
pH Changes in Healing Fractures.

Time after fracture			2½ Hrs.	24 Hrs.	3 Days	5 Days	7 Days	9 Days	11 Days	13 Days	15 Days
pH	Hematoma	220-43	7.18	7.07	7.19	7.22	7.29	7.36	7.32	7.47	7.53
,,	,,	217-43	7.42	7.33	7.26	7.28	7.29	7.51			
,,	,,	235-43	7.34	7.25	7.37	7.42	7.52				
,,	,,	215-43	7.28	7.25	7.29	7.24	7.51	7.46			
,,	,,	309-43	7.35	7.08	7.24	7.31	7.41				
,,	,,	333-43	7.36	7.36	7.25	7.30	7.41	7.54			
,,	,,	3-44	7.15	7.13	7.30	7.34	7.44	7.54			
,,	,,	127-43	7.30	6.90	7.02	7.23	7.42	7.60			

Lean.[1,2,3] Less complete biochemical knowledge of fracture repair is available. Clinicians are interested in the process of calcification and ossification, as they are at a loss at times to explain nonunion of fractures or to alter the clinical course of this complication without extensive surgical procedures.

Detection of any postulated biochemical disturbance in calcification in nonunion presents an almost insurmountable problem. First of all, little is known of the biochemistry of normal fracture repair. Most investigations of fracture healing have been morphological studies. Correlation of the biochemical changes with the morphological changes would be interesting and instructive. Analyses of tissue in the region of healing fractures have been made. However, in the early stages of repair there is only a liquid hematoma at the fracture site and tissue analysis is impossible. The presence of the hematoma is fortunate in one respect in that it is comparatively simple to draw out fluid and subject it to chemical analysis. While the hematoma does not represent accurately the biochemical processes in early fracture repair, it probably reflects these factors sufficiently to yield reliable information concerning changes in concentration of various substances as preparations for calcification take place. Assuming that this is true, aspiration of the hematoma and analysis of the fluid is a relatively simple technic for biochemical study of fracture repair. A major problem is the small volume of hematoma fluid present at the site of fracture necessitating the use of microchemical methods.

Determination of the hydrogen ion activity of the hematoma at fracture has been made by Murray,[4] Sterling,[5] and Greune.[6] Technical difficulties seriously limited the number of determinations that were made. Murray and Sterling generally agree, both reporting a pH on the acid side initially, with subsequent change to the alkaline side. In several experiments Greune found a tendency of the fracture site to remain on the acid side. No mention is made of any evidence of infection in these fractures. It was our experience in all instances that persistent acidity at the fracture was accompanied by infection. A desire to determine the timing and extent of pH changes during fracture repair prompted these experiments.

Methods. The first problem was to produce a fracture with a hematoma of adequate size, which remained fluid sufficiently long to permit repeated aspirations for pH determinations. Numerous trials established that the best method to produce such a fracture in a canine femur was to elevate a full thickness spicule of bone and excise a portion of the vastus lateralis muscle, thus providing room for an ample hematoma. Heparin was used in some hematomas in the hope of maintaining a more prolonged fluid state. This technic was of little avail and heparin was not used in the later experiments.

Aspiration of the hematoma was done with 16 gauge needles, having multiple perfora-

[1] Urist, M. R., and McLean, F. C., *J. Bone and Joint Surg.*, 1941, **23**, 1.

[2] Urist, M. R., and McLean, F. C., *J. Bone and Joint Surg.*, 1941, **23**, 283.

[3] Urist, M. R., *J. Bone and Joint Surg.*, 1942, **24**, 47.

[4] Murray, C. R., *Indust. Med.*, 1941, **10**, 171.

[5] Stirling, R. I., *Tr. Med. Chir. Soc. Edinburgh*, pp. 203-228, 1931-1932; in *Edinburgh M. J.*, Dec. 1932.

[6] Greune, H., *Deutsche Ztschr. f. Chir.*, 1931, **203**, 324.

tions of the wall near the beveled end. Only rarely did infection of the fracture follow. Most infections seemed to be caused by contamination at operation as the signs of infection appeared early. Late infections attributable to repeated aspirations were rare. Many of the fractures made were unsuitable for study, as the amount of hematoma fluid was insufficient to yield fluid by repeated aspirations for the 7 to 15 days necessary for calcification, demonstrable in a biopsy of the fracture site. Small hematoma and infection made many of the fractures produced unsuitable for study.

Aspiration of the hematoma was carried out 2½ hours after the fracture was produced, again 24 hours later, then every 48 hours until fluid was unobtainable. pH determinations were made in duplicate by means of a micro-glass electrode, designed by one of us—(C. L. C.), capable of making readings on as little as 0.008 ml of fluid. Biopsies were taken at various intervals to correlate the morphology with the pH changes, and to check specifically for beginning calcification by the Kossa staining technic.

Results. The first aspirations, performed 2½ hours after the fracture was produced, yielded a bloody fluid with a pH more acid than that of the venous blood. In a series of 8 fractures, (2½ hours after the fracture was produced) the average pH of the fluid was 7.30, and 24 hours later, the pH in the 8 fractures averaged 7.18. No analysis was carried out to try to determine the substances responsible for the local acidity.

A fracture becomes the center of an intense acute non-infectious inflammatory reaction and, according to Menken,[8] the pH initially in such a reaction should be definitely acid. Breakdown of tissue, damaged at the time of fracture, with more or less isolation of the site of injury from the general circulation, contributes to the local acidity.

As repeated aspirations were performed, at 48-hour intervals, the fluid gradually changed to the alkaline side. In the 8 fractures the average pH at the end of the experiment was 7.52. The shift to the alkaline side, as repair takes place, is difficult to explain. Certainly as the site of injury is vascularized, the local acidity is neutralized. However, this does not explain the peculiar local change to the alkaline side.

The end of each of the 8 experiments was determined by inability to aspirate sufficient fluid for pH determinations and varied from 7 to 15 days. Biopsy of the fracture site was carried out at the termination of each experiment and in 6 out of 8 there was calcification demonstrable by the Kossa staining technic. The material from the remaining two experiments showed questionable calcification. Six other fractures were biopsied from 3 to 5 days after fracture when the pH of the hematoma was still on the acid side or about the same as the venous blood and in none of these was it possible to demonstrate calcification.

The rôle of the hydrogen ion concentration in calcification and ossification is difficult to ascertain. A major rôle has been ascribed to it by the exponents of a theory of calcification.[4,5] This postulates that there is initially a high concentration of calcium ions at the fracture site, due to the increased solubility of the bone calcium salts in the acid hematoma fluid. Actually no measurements of calcium have been reported confirming this theory. As the calcium salts are less soluble in an alkaline solution, it is further postulated that precipitation of calcium takes place as the hematoma at the fracture site becomes alkaline and repair progresses. Certainly such changes in the pH do take place in the hematoma. However, calcification is probably a more complicated mechanism than this theory sets forth. These changes in pH values may be results rather than the controlling factor in calcification.

A persistently low pH at the fracture site invariably indicated infection. It was possible to detect an infected fracture by the persistence of low pH readings before local signs of infection developed. As long as local infection persisted, it was impossible to

[7] Claff, C. L., and Swenson, O., *J. Biol. Chem.*, 1944, **152**, 519.

[8] Menkin, V., *Am. J. Path.*, 1934, **10**, 193.

demonstrate calcification until the inflammation subsided.

Conclusions. 1. In the dog, there is an initial local acidity in the hematoma resulting from a fracture, followed by a change to alkalinity beyond the value in the venous blood.

2. Biopsy of the fracture site after the fluid became alkaline, at the end of the experiment, showed calcification in 6 out of 8 cases.

3. Infection at the site of fracture produced a persistent local acidity.

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Granulocytopenia in Rats Given Thiourea and Thyroxin. The Therapeutic Effect of *L. casei* Factor.

FLOYD S. DAFT, ARTHUR KORNBERG, L. L. ASHBURN, AND W. H. SEBRELL.

From the Division of Physiology and the Pathology Laboratory, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

The effect of thiourea and its derivatives in depressing the functional activity of the thyroid gland in experimental animals and in man is well known and the literature pertaining to the discovery of this phenomenon has been repeatedly reviewed.[1,2] In the first report on the clinical use of thiourea and thiouracil in the treatment of thyrotoxicosis[1] there was noted the development of agranulocytosis in one patient. A number of additional cases of leucopenia, granulocytopenia or agranulocytosis following the use of these drugs have been described. The importance of finding a method for preventing the development of these blood dyscrasias is obvious. It appeared that there might be an analogous situation in the occurrence of granulocytopenia in rats fed sulfonamides in purified diets[3,4] and the correction of this dyscrasia with *L. casei* factor.[5] It was decided, therefore, to study the effect on rats of administering thiourea in a similar purified diet with and without the simultaneous administration of thyroxin or thyroid powder. If granulocytopenia developed it would then be possible to test the therapeutic value of *L. casei* factor in these animals.

Anemia without granulocytopenia has been encountered in many of the animals receiving thiourea without thyroxin or thyroid powder. Granulocytopenia without anemia has occurred in high incidence in animals receiving both thiourea and thyroxin or thyroid powder and the granulocytopenic animals have been treated successfully with *L. casei* factor.

Experimental. The basal (control) diet used in these investigations consisted of leached and alcohol-extracted casein 18%, Crisco 8%, modified Osborne and Mendel salt mixture[3] 4%, $FeSO_4.7H_2O$ 0.18%, $CuSO_4.5H_2O$ 0.02% and anhydrous dextrose (Merck's U.S.P.) 69.8%. Into each 100 g of diet were incorporated 1 mg of thiamine hydrochloride, 2 mg of riboflavin, 2 mg of calcium pantothenate, 1 mg of pyridoxine hydrochloride and 200 mg of choline chloride. Each rat received a supplement twice weekly of 0.25 cc of corn oil containing 2000 units of vitamin A and 200 units of vitamin D (Natola). The experimental diets differed only in that specified ingredients were substituted for equivalent amounts of dextrose. Diet No. 985 contained 1% of thiourea; diet No. 1053 contained 1% of thiourea and

1 Astwood, E. B., *J. Am. Med. Assn.*, 1943, **122**, 78.

2 Mixner, J. P., Reineke, E. P., and Turner, C. W., *Endocrinology*, 1944, **34**, 168.

3 Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep.*, 1942, **57**, 1559.

4 Kornberg, A., Daft, F. S., and Sebrell, W. H., *Science*, 1943, **98**, 20.

5 Daft, F. S., and Sebrell, W. H., *Pub. Health Rep.*, 1943, **58**, 1542.